VOLUME LXXVII NUMBER 1

THE

BOTANICAL GAZETTE

March 1924

FERN RUSTS OF ABIES

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(WITH PLATES I–V AND TEN FIGURES)

Introduction

The fern rusts of *Abies* include most of the species of the genus *Uredinopsis*, and possibly at least one species of *Hyalopsora*. There are a number of other rusts that parasitize *Abies*, representing a number of distinct genera; but those of *Uredinopsis*, the main theme of the present research, are the most frequent and perhaps the foremost in interest and importance. This is certainly true in the case of *Abies balsamea*.

Many problems have presented themselves during the course of the investigations of this genus, but among them three types have proved to be especially attractive. First, there are many unsolved problems regarding structure, sporogenesis, and phylogeny. A second complex deals with the phenomena of heteroecism, biological strains, and hence the exact delimitation of species. A third group calls for recognition, namely, a determination of the amount and the effect of the injury caused to their hosts by the various species of *Uredinopsis*, for not infrequently they are responsible for extensive damage to the new foliage of balsam firs, particularly the younger trees, and one species, newly described, modifies the conformation of its host. It is to the first set of problems that most attention has been given, but the others have not altogether been overlooked.

While *Uredinopsis* is the principal subject matter of these studies, a limited place has been given to *Hyalopsora*, since infections of *Hyalopsora* on one of the fern hosts were so frequently encountered, in such close association with *Uredinopsis*, that they appeared to have a bearing on certain of the *Uredinopsis* problems. The results in this connection have exceeded expectations, for through the discovery of a second new *Peridermium* on *Abies*, and preliminary inoculation experiments, there is now good reason to believe that *Hyalopsora* should be listed among the fern rusts of balsam. If so, a new field of inquiry is opened up.

The materials for this research were obtained mainly from the Timagami Forest Reserve in the Province of Ontario, a forest area of upward of 6,000 square miles, lying about 300 miles north of Lake Ontario, and one that is richly covered with the balsam fir.

Taxonomy

Uredinopsis is one of the three genera of rusts known to parasitize ferns. It was founded by MAGNUS (**18**) in 1892 on the type species *U. filicina* (Niessl) Magnus. He studied this fungus (a form then known as *Protomyces? filicinus* Niessl) as it occurs on *Phegopteris vulgaris* Metten. and discovered that in addition to the so-called "stylospores," since shown to be urediniospores, there were hyaline spores imbedded in the leaf parenchyma. These he called endospores, and because of the existence of this additional spore form he placed the fungus in a new genus, which he called *Uredinopsis*, and he renamed this species *U. filicina* (Niessl) P. Magnus. In 1895 STÖRMER (**23**) observed and reported a second *Uredinopsis* on *Onoclea Struthiopteridis* (L.) Hoffm., which he found in Norway near Christiania, and named it *U. Struthiopteridis*.

It remained for DIETEL (**6**), however, to discover that the fungi placed in this new genus *Uredinopsis* were rusts. In 1895, while working on *U. Struthiopteridis* Störmer, he germinated the hyaline spores discovered by MAGNUS, and found that they gave rise to the typical four-celled promycelia of the Uredineae. Thus these spores were demonstrated to be teliospores, and the fungi producing them were accordingly classified as rusts. DIETEL also described the two known European species of *Uredinopsis*, *U. filicina* (Niessl)

Magnus and *U. Struthiopteridis* Störmer. He also described and named *U. Pteridis*, a rust on *Pteris aquilina*, which had been sent to him from California.

Up to this time the European mycologists were not aware that PECK had antedated them (1872) by describing a parasite on *Onoclea sensibilis* L. which later was recognized to be a *Uredinopsis*. PECK interpreted this fungus as one of the Fungi imperfecti, and named it *Septoria mirabilis*. In 1904, MAGNUS, after reviewing FARLOW and SEYMOUR'S list (**9**), and a more recent publication by H. and P. SYDOW (**24**), in which they described a supposedly new form, *U. americana* (Sydow) on *Onoclea sensibilis*, re-named PECK'S fungus *U. mirabilis* (Peck) P. Magnus, and interpreted *U. americana* Sydow as a synonym. He also reported that he had received from America, through Professor G. F. ATKINSON, specimens of two new species of *Uredinopsis*. One, on *Aspidium Thelypteris* (L.) Sw., he named *U. Atkinsonii;* the other, on *Osmunda cinnamomea* L., he named *U. Osmundae*.

About a month before the publication of the work by MAGNUS, SYDOW (**25**) had published the description of another species for America, *U. Copelandi* on *Athyrium cyclosorum* Rupr. Three years later, ARTHUR (**2**) published a complete list of the North American species of *Uredinopsis*, and included in it still another new species which he had found on *Phegopteris Dryopteris* (L.) Fee, and which he named *U. Phegopteridis*. In 1906 ARTHUR (**1**) had substituted for *Uredinopsis* the name *Milesia* of WHITE (**29**), but he immediately abandoned this name, for, as stated by GROVE (**14**), "the genus *Milesia* is now dropped, because it was founded on an imperfect state which might belong to any one of several genera." ARTHUR'S list includes seven species: *U. mirabilis*, *U. Struthiopteridis*, *U. Osmunda*, *U. Atkinsonii*, *U. Phegopteridis*, *U. Pteridis*, and *U. Copelandi*. Of these, *U. Struthiopteridis* is found in both Europe and America, and the remainder in America only; *U. filicina* is apparently restricted to Europe.

The genus as a whole was so far described as occurring solely on ferns and possessing teliospores and two other spore forms. The main features in the descriptions can be summarized as follows. Teliospores, of one to four or more cells, were described as being

scattered throughout the mesophyll. One of the other spore forms was always recognized as being thin-walled, colorless or white in mass, fusiform, beaked, and usually appendaged. This form was supposed to be borne singly on pedicels, and was observed and reported by all investigators. The third form was not reported for all species; it was illustrated as thick-walled, irregularly oval, and borne singly on pedicels. The identity of the teliospores had been proven by DIETEL (**6**). The fusiform spores germinated readily and immediately in a drop of water, and were recognized by all as urediniospores, but the function of the thick-walled spores was not understood. They had not been germinated except in one instance by DIETEL, who called them single-celled teliospores. MAGNUS considered them a second form of urediniospore; and ARTHUR, for lack of a better name, described them tentatively as aeciospores.

In 1913–14 considerable advance was made in the knowledge of the life history of *Uredinopsis* by FRASER (**12, 13**). He carried out inoculation experiments on the five species, which he had reported for Nova Scotia in 1910 (**10**), namely, *U. mirabilis*, *U. Struthiopteridis*, *U. Osmundae*, *U. Atkinsonii*, and *U. Phegopteridis*, and demonstrated that *Peridermium balsameum* Peck was the aecial stage of these rusts. This was the first announcement that *Uredinopsis* is characterized by heteroecism. In 1917 WEIR and HUBERT (**27**) made a further advance by connecting a peridermium on *Abies grandis* Lindl. and *A. lasiocarpa* Nutt. with *Uredinopsis Pteridis* on *Pteris aquilina* L. (*Pteridium aquilinum pubescens*).

Some confusion unfortunately has arisen regarding the identity of the *Peridermium* which WEIR and HUBERT referred to as *Uredinopsis Pteridis*. This is due apparently to lack of experimentation; they now regard the *Peridermium* as something new. JACKSON (**15**), however, claims that it is identical with *Peridermium pseudobalsameum* (D. and H.) ARTHUR and KERN (**3**), and lists it as such. In absence of evidence to the contrary, I shall follow JACKSON's example and refer to it as *Peridermium pseudo-balsameum*.

A further difference of opinion has arisen in regard to the identity of the previously described *Peridermium balsameum* and *P. pseudo-balsameum*. WEIR and HUBERT claim that they are

the same, but it should be noted that there appear to be certain decided differences between the two. Thus, in *P. balsameum* the thickness of the aeciospore wall is 1–1.5 μ, and the pycnia are "in section hemispherical, 100–130 μ broad, 30–50 μ high"; whereas in *P. pseudo-balsameum* the thickness of the aeciospore wall is 2.5–3.5 μ, and the pycnia are "globose, large, 160–175 μ broad" (3). If, in addition to these differences, *P. pseudo-balsameum* attacks the second year needles, there should be no difficulty in distinguishing between these two, for in the east *P. balsameum* attacks the first year needles only. In this case there could not be much doubt that the *Peridermium* on the second year needles which WEIR and HUBERT associated with *Uredinopsis Pteridis* is really *P. pseudo-balsameum*.

There still remains the question of the life cycle of *Uredinopsis Copelandi*. On the basis of morphology only, WEIR and HUBERT consider that it should be regarded as a synonym of *U. Pteridis*. On the other hand, from observations made on the distribution of *U. Copelandi* in the field, JACKSON concludes that it should be connected with *Peridermium balsameum* on *Abies grandis* and *A. nobilis*. It is impossible to decide between these conflicting views until the life history of *U. Copelandi* has been worked out by means of inoculation experiments.

After reviewing the work of these investigators, the life histories of the species of *Uredinopsis* as demonstrated by inoculation can be summarized as follows. *U. mirabilis*, *U. Struthiopteridis*, *U. Osmundae*, *U. Atkinsonii*, and *U. Phegopteridis* have stages II and III on various species of ferns, but for each its own special set of hosts, and stages O and I (as *Peridermium balsameum*) on *Abies balsamea*. *U. Pteridis* has stages II and III on *Pteris aquilina* (*Peridermium aquilinum pubescens*), and stages O and I (as *Peridermium pseudo-balsameum*) on *Abies grandis*, *A. amabilis*, and *A. lasiocarpa*. *U. Copelandi* has stages II and III on *Athyrium cyclosorum*, but I have not been able to find any record of experimental work connecting this species with an aecial stage.

Reference should next be made to discussions among various students of the rusts regarding the individuality of the species of *Uredinopsis* as listed by ARTHUR. This subject involves the ques-

tion of morphological species as contrasted with biological species, a distinction which has been overlooked in many of the conclusions reached. FRASER appears to be the only one who has approached this matter from an experimental point of view. In 1913 (**13**) he carried out cross inoculations with species of *U. mirabilis*, demonstrating that the spores of this species would infect its own fern host only, namely *Onoclea sensibilis*, and that other ferns remained free even after repeated attempts and most careful inoculation. He considered this sufficient evidence on which to establish *U. mirabilis* as a distinct species. His statement on the subject is as follows: "These experiments confirm the work of last year and indicate clearly that *Uredinopsis mirabilis* is a distinct species." This is as far as FRASER went. Another statement, to the effect that "the species of the genus *Uredinopsis* are not separated by any marked morphological differences" (**13**), has left him open to a misunderstanding on the part of some recent writers. Thus WEIR and HUBERT state: "FRASER in his last article came to the conclusion that all five species with which he had been working were identical with the exception of *U. mirabilis*." On reviewing FRASER'S papers, I could not find any statement which could strictly be interpreted in that way. The latter quotation from FRASER appears to be his only pronouncement on the subject, and in that he certainly does not go as far as WEIR and HUBERT suggest.

The last two investigators have made an extensive comparison of the sizes of the spores of *Uredinopsis*. After examining the measurements they obtained, and reviewing the publications of ARTHUR and FRASER, they conclude that on morphological grounds all the species of *Uredinopsis* should be grouped in two divisions, namely, *U. mirabilis*, with *U. Struthiopteridis*, *U. Osmundae*, *U. Atkinsonii*, and *U. Phegopteridis* as synonyms; and *U. Pteridis* with *U. Copelandi* as a synonym. A different grouping of one of these species is suggested by JACKSON (**15**). After a comparison of morphological characters, he concludes that *U. Atkinsonii* is the same as *U. Copelandi*. The idea of combining species is carried to the extreme by RHOADS, HEDGCOCK, BETHEL, and HARTLEY (**22**), who record only one species of *Uredinopsis*, namely, *U. mirabilis*,

and list all others named by ARTHUR as synonyms. They quote FRASER and WEIR and HUBERT in supposed support of this idea.

It appears to me to be still premature to recast the species of *Uredinopsis* as given by ARTHUR. There are questions which should first be solved, and also certain physiological and morphological differences that cannot be overlooked. For instance, the aecial stage of *U. Copelandi* has never been demonstrated by inoculation experiments, and yet such work is necessary before any statement can be made regarding the complete life history of this species. Also, WEIR and HUBERT point out that, as the aecial stage of *U. Pteridis* attacks the second year needles and not the young ones, the life history of this rust may be decidedly different from such a species as *U. mirabilis*, the aecial stage of which attacks young needles only. They suggest that the teliospores of *U. Pteridis* germinate in the late summer, and that the rust overwinters as mycelium in the *Abies* leaf. This may or may not be the correct explanation. In any case, the fact that the age of the needles attacked varies from species to species and yet is constant within each species, indicates the existence of a marked physiological difference. Of the forms which have come under my observation in the Timagami Forest Reserve, *U. Phegopteridis* occurs in great profusion early in the season, and lasts on the fern throughout most of the summer. *U. Atkinsonii* can also be found throughout the summer, but it occurs in small quantities only; *U. mirabilis* and *U. Osmundae* appear in large quantities during the last of July and August and mature rapidly. Of these two *U. Osmundae* is the more common. For the locality mentioned these conditions are constant. As for the morphology of these rusts, minor but constant variations are just as pronounced. The difference between the thicknesses of the aeciospore wall of *Peridermium balsameum* and *P. pseudo-balsameum* has already been noted. In *U. Osmundae* the layer-like character of teliospores is more highly developed than in any other species. This shows clearly in figs. 23 and 24. This feature of *U. Osmundae* has been noted by many investigators and is constant for the species. Other minor differences have been noted, such as extent of spore production, size of pustule, color of infected area, etc., and although these

differences are very small, yet they appear to be constant. Thus the evidence would indicate that the number of species as given by ARTHUR should certainly not be reduced.

Spore forms of Uredinopsis

FRASER and WEIR and HUBERT in their experimental work demonstrated that all six of the species of *Uredinopsis* investigated are heteroecious, and that they produce all the ordinary rust spore forms, pycnospores, aeciospores, urediniospores, and teliospores. In addition to these, as already noted, MAGNUS called attention to single-celled thick-walled spores found in pustules along with the uredinia in certain species. From a histological point of view very little that is new has been observed with reference to the aeciospores; the greatest interest attached to them is in the times of their appearance for the different species, and in the ages of the needles on which they occur. On the other hand, the pycnia are of distinct interest, especially in a comparative study of the pycnia rust types found on the needles of *Abies*. An account of these is given with the description of new species in the latter part of this paper. An extended study has been made of the other spore forms, detailed accounts of which follow.

SINGLE-CELLED THICK-WALLED SPORES

Reference has already been made to the diversity of views held with regard to the single-celled thick-walled spores described by MAGNUS. They have been variously interpreted as resting urediniospores, teliospores, and aeciospores, but, so far as I can find, no record exists of any demonstration of their function by means of inoculation tests. As a matter of fact, neither their identity as *Uredinopsis* spores nor their function has ever been proved.

Unlike the appendaged fusiform urediniospore, this thick-walled form has not been reported for all species of *Uredinopsis*. Of the seven rusts of this genus listed by ARTHUR, the thick-walled spores are "unknown" in the case of three, *U. Osmundae*, *U. Pteridis*, and *U. Phegopteridis*. Also investigators have reported this spore form as missing even from examples of species where it was supposed

to occur. For instance, FRASER (12), who must have collected and examined a large amount of material, states that "the spores, which have been regarded as aeciospores, were rarely present in the collections." In my own experience I have repeatedly hunted for these spores on *Onoclea sensibilis*, *Osmunda claytoniana*, and *Asplenium felix-foemina*, but without finding them in a single instance, although the ferns were heavily infected with their respective species of *Uredinopsis* and showed an abundance of fusiform urediniospores and teliospores. It is significant also that since the publication of the work of FRASER connecting *Uredinopsis* on the fern with an aecial stage on the balsam, the literature contains practically no mention of this thick-walled spore. The references to it are found chiefly in the earlier articles written by MAGNUS and DIETEL, and these investigators must have worked chiefly with dried material.

The persistence of DIETEL and MAGNUS in insisting on the existence of these spores as a phase in the life history of *Uredinopsis* is explainable in one of three ways: (1) that they do occur in certain species growing in certain localities, but not at all or irregularly in others; (2) that they are atypical urediniospores borne in young or suppressed pustules; and (3) that they really belong to some other species of rust simultaneously parasitizing the same host as the *Uredinopsis*. My experience would suggest that the third explanation is worthy of consideration.

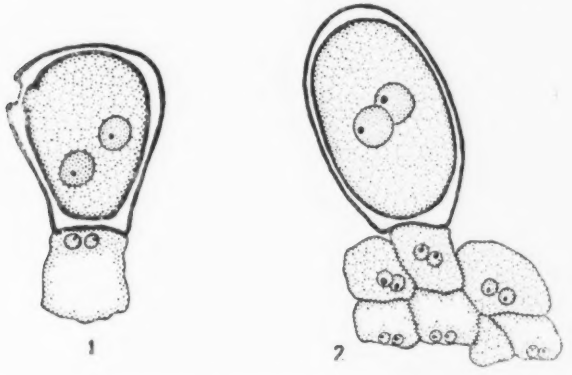
I have observed thick-walled spores produced in great abundance on *Phegopteris Dryopteris*. In some cases small quantities of these spores were associated with rich productions of the fusiform urediniospore of *Uredinopsis Phegopteridis*. In such instances the thick-walled spores conformed to the spore type referred to *Uredinopsis*, and they were borne chiefly in unbroken pustules. At times they appeared to be coming out of the same pustule as the white filaments of the beaked fusiform urediniospores, but on close examination and sectioning, it was always found that the pustules of the two kinds were really separate. In the same district the *Phegopteris Dryopteris* was infected with *Hyalopsora Aspidiotus* (Peck) Magn. This was compared with *H. Polypodii-dryopteridis* Magn. (*H. Aspidiotus*) no. 2395 of the Sydow

Exsiccati. The two were found to be identical. This same *H. Aspidiotus* was then in turn compared with the thick-walled spores found with the *Uredinopsis*, and these two also were identical. On studying the distribution of the two spore forms in question, I found three conditions, as follows: (1) ferns infected with only the fusiform appendaged spores; (2) ferns infected with only the oval thick-walled spores; and (3) ferns infected with both spore forms. These three conditions often occurred close together, and also each condition was found by itself, and often some distance from patches of fern infected in either of the other ways.

To test this matter experimentally, on July 20, 1921, I obtained four boxes of *Phegopteris Dryopteris* plants, free from disease, with from two to four plants in each box. None of the ferns in the vicinity from which these ferns were collected was infected with *Uredinopsis* or *Hyalopsora* at that time or at any other time during the summer. I inoculated the ferns in two boxes with the beaked fusiform urediniospores, and kept the ferns in the other two boxes as controls. The spores used for these inoculations were taken from ferns carrying both kinds of spores. On July 29 pustules giving off white filaments of the beaked fusiform urediniospores had developed on the inoculated ferns; but pustules of the thick-walled spores did not develop either then or later. The controls remained free from any infection.

While examining and identifying the spores of *Hyalopsora* and the thick-walled spores found with the *Uredinopsis*, I was at first at a loss to know whether they were stalked or sessile. From the descriptions of these spores in the manuals, it appeared to be necessary to decide this point before making a satisfactory identification. In mounts made from scrapings of either *Hyalopsora* or the thick-walled type associated with the *Uredinopsis*, most of the spores had no stalk; but occasionally spores were observed with an apparent stalk attached. Also in hand sections most spores appeared to be sessile, but here and there a spore would appear to be borne on a pedicel. The literature on this subject is contradictory. ARTHUR (2) describes both the spores of *Hyalopsora* and the so-called thickwalled spores of *Uredinopsis* as being borne on pedicels. GROVE (14) describes those of *Hyalopsora* as sessile,

and those referred to *Uredinopsis* as stalked. On the other hand, BARTHOLOMEW (**4**) has illustrated a sorus of *Hyalopsora* containing spores borne on fairly long stalks. My own studies on this feature were based on microtome and hand sections from carefully killed and fixed material. In the sections made they appear as illustrated in text figs. 1 and 2. The spore is the terminal cell of a row, and if these basal sterile cells come away with the spore, they look like a stalk. Of course, any increase in the spaces between these rows of basal cells invariably accentuates this stalklike appearance.



FIGS. 1, 2.—Urediniospores of *Hyalopsora Aspidiotus*, showing basal or stalklike cells; ×953.

Thus BARTHOLOMEW, who used a dissecting and teasing-out method, reports that the spores are always borne on pedicels. In the same way, the slightest deterioration in material (such as would be caused by a strong killing fluid), a slight delay between time of collecting and killing, and of course drying, causes these basal cells to collapse and assume more and more the appearance of specialized stalks, and it will be recalled that many of the original descriptions were made from dried plants. In most preparations, however, in whatever way made, these rows of cells form a compact mass at the base of the pustule, and the spores appear to be sessile. Thus although the thick-walled spores associated with *Uredinopsis*, in regard to their stalks, have been described differently from the urediniospores

of *Hyalopsora*, I found them to be identical in this respect. Whether they should be considered as stalked or sessile remains an open question.

Still another difficulty arose in regard to the color of the thick-walled spore, and here also the descriptions in the manuals differ. Although these spores often have a distinct and characteristic shade, too much reliance cannot be placed on this point, especially in cases where the description states the color in terms which do not permit any variation, for I have found yellow and colorless thick-walled spores in the same pustule. Also, on certain material which was collected, pressed, and dried, the spores when fresh were a glistening golden yellow, but some months afterward, when thoroughly dried, most of the spores showed very little color, and many were absolutely colorless. In all cases observed the wall was always colorless. Thus again we find an instance in which the drying of the plants used in making the original descriptions, or perhaps a difference in the age or preparation of specimen, may account for the fact that these spores were sometimes described as yellow, and sometimes as colorless.

This evidence suggests that the thick-walled spores attributed to *Uredinopsis* in some instances at least have been really spores of *Hyalopsora;* and in cases where they were reported as being found with a *Uredinopsis* spore form, the host was really infected with two distinct organisms. Of course, infection work, especially with the reputed thick-walled spores of *Uredinopsis*, will be necessary to really prove this.

Before leaving this subject a brief comment should be made on two suggestions made by German investigators. First they laid great stress on the fact that the thick-walled spores are often borne in unbroken pustules, and on these grounds they conclude that the thick-walled spore might be a suppressed form of the beaked urediniospores. I have also observed a large number of unbroken pustules of these spores (fig. 21), and found them associated chiefly with a predominant growth of *Uredinopsis* and a rich production of the fusiform urediniospores of that genus. No difference could be detected, however, between the thick-walled spores in the unbroken pustules and those in the pustules which

were broken open and discharging their spores, except that the spores in the unbroken pustules were often smaller and immature. Likewise, I do not find that there is any gradual transition between the thick-walled, oval spores and the thin-walled, beaked, fusiform urediniospores. I have examined unbroken pustules of each, and the many characters which clearly separate the mature spores can easily be detected in the young pustules, provided the proper technique is employed. Of course, each form varies so greatly that, if the young growths were examined on dried or poorly preserved material, the changes from the normal form might be such that the two kinds of spores and the pustules in which they are contained would appear to be very much alike.

Secondly, if, as MAGNUS suggested, this thick-walled spore is a second form of *Uredinopsis* urediniospore, one might expect the general characters of the two forms to be more alike than they are. For instance, the beaked fusiform spores are consistently hypophyllous, whereas the thick-walled oval spores are amphigenous. In fact, the latter are borne in a very irregular manner. Instances were observed in a number of different pustules where spores were borne on both sides of the mycelial mat. Fig. 27 shows this condition. In the section photographed only one spore shows on the inner side, but there were several in the other sections of the series through this pustule. In one striking case a spore had developed in the center of the mycelial mat at the base of the pustule (fig. 28). No such irregularity is found in the distribution of the beaked fusiform urediniospore.

UREDINIOSPORES

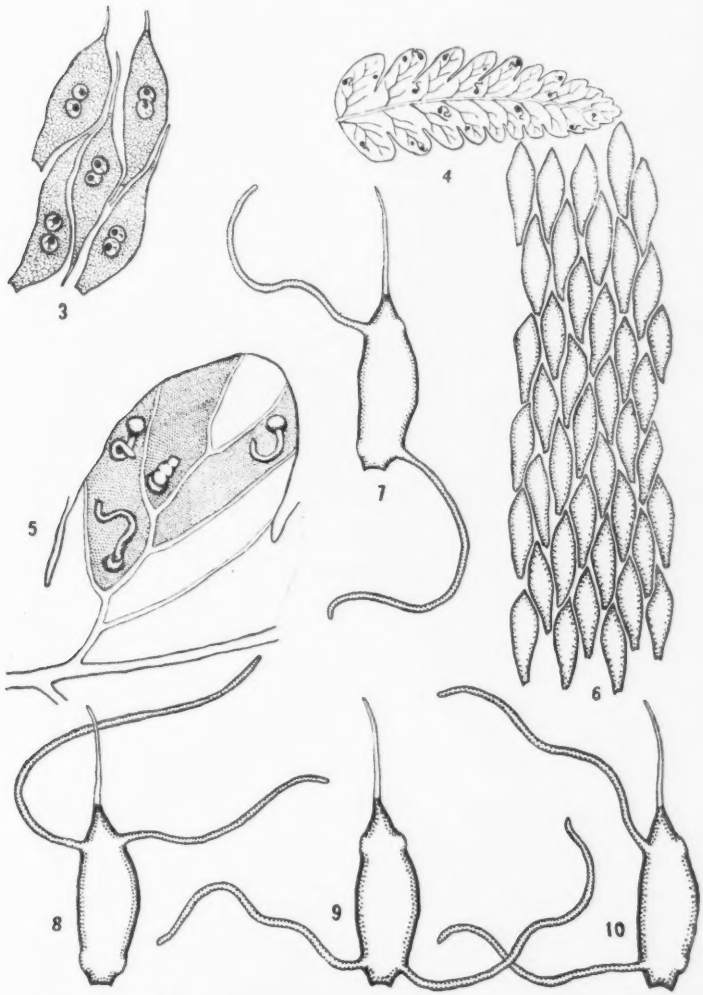
The urediniospores of *Uredinopsis* are unique in form and in method of discharge. They are large, colorless, fusiform, single cells, usually appendaged, and, being slightly adhesive, emerge from the pustules in which they are produced in narrow ribbon or tendril-like masses, quite after the manner of the conidia of many of the imperfect fungi. The uredinial pustules are lined by a peridium consisting of binucleate cells, and the floor is made up of a mass of interlacing threads divided into binucleate cells; from this basal pad the binucleate spores originate. Hitherto the

urediniospores have been supposed to arise singly and not catenulately, and on this ground the genus finds its place in the accepted systems of classification. In my studies of these spores special attention has been given to their mode of origin, supplementing by comparative studies on other suggestive forms; incidentally observations have been made on their markings and mode of germination. The only histological work which has been done on *Uredinopsis* is that of DIETEL (**6**) and MAGNUS (**19, 20**), apparently using hand sections and dried material only. They observed the peridium, but did not recognize the method by which the urediniospores were produced. They believed that these spores were borne singly on the floor of the uredinium, a conclusion not to be wondered at when the imperfections of the material used are taken into consideration.

METHOD OF PRODUCTION.—The species examined in my studies of urediniospore production are *U. mirabilis*, *U. Osmundae*, *U. Atkinsonii*, and *U. Phegopteridis*. The last named rust, which parasitizes *Phegopteris Dryopteris*, proved to be best adapted for this purpose. Figs. 1–15 and text figs. 3–10 were all drawn from specimens of that species. The material was collected in the Timagami Forest Reserve, Ontario, during the summers of 1919, 1921, and 1922. Some of it was killed in picro-sublimate and some in Flemming's weak chrom-acetic osmic solution. It was imbedded in paraffin, cut in sections 7 μ thick, and stained with iron alum haematoxylin, and either erythrosin or acid fuchsin as counter stains.

In collecting this material, I found it advisable to obtain it after a period of dry weather. The spores under such conditions escape from the sorus in a streamlike manner, often forming quite a long curved threadlike mass (text figs. 4, 5). This white thread or ribbon is seen through the microscope to be a mass of spores closely packed together; when flattened out slightly under the cover-glass, it appears as it is in part illustrated in text fig. 6. During a rain storm the spore tendrils and the mature spores in the pustules become dispersed, and a collection made at such a time may include many empty pustules.

In sections prepared in this way the development of an individual spore can usually be followed quite clearly. At first it is a roundish,



FIGS. 3–10.—Fig. 3, five immature urediniospores of *Uredinopsis*, illustrating peculiar shapes assumed during development; ×827. Fig. 4, pinna from frond of *Phegopteris Dryopteris*, illustrating uredinia and method of urediniospore discharge; ×2. Fig. 5, single pinnule of *P. Dryopteris*, showing uredinia and method of urediniospore discharge (infected leaf islets darkened); ×20. Fig. 6, portion of one of tendrils of discharging urediniospores as it appears under microscope when crushed out under cover glass; ×315. Fig. 7, germinating urediniospore of *Uredinopsis*, germ tubes coming from diagonally opposite pores; ×827. Fig. 8, germinating urediniospore of *Uredinopsis*, both germ tubes coming from pores at apical or distal end; ×827. Fig. 9, same, with both germ tubes coming from pores at basal or proximal end; ×827. Fig. 10, same, with both germ tubes coming from pores on one side of spore; ×827.

binucleate cell in the outer portion of the matted hyphae at the base of the sorus (figs. 1–3). This cell elongates and divides into two (figs. 4–6). The outer one elongates further and the inner one continues to divide. As these cells are cut off in this catenulate fashion, they at once begin to develop the fusiform shape of the mature spores. At the same time the connections between them become very narrow and delicate, and, as the spores mature, it is often impossible to distinguish the connecting walls. Figs. 8–11, showing the gradual development of the spore, were drawn from examples where the connection could still be distinguished. It is not usual to find more than three cells in an unbroken row. By this time the distal cell has nearly reached its complete development as a spore, and becomes detached by breaking the delicate wall connecting it with the cell immediately following (fig. 7). The remnant of the broken connection can be seen at the base of nearly all mature spores (figs. 12–15).

In a mature sorus so many separated spores become packed together that their catenulate origin would not be suspected except after a very close examination of the base of the pustule, and this is the stage at which the rust is usually collected (fig. 2). In a young sorus, when the spores are not fully developed, the catenulate formation is at once evident (fig. 1).

During the early stages of development, the spores appear to be very much crowded together, and often assume unusual shapes (text fig. 3). This crowding, and the narrowing of the connection between the cells, may play a part in the formation of the beak and appendage. That is, if the connection becomes drawn out and very narrow, and then breaks away from one spore, it may form an appendage for the other spore. This is also suggested by the fact that spores have often been observed with two appendages, one at each end. Others have an appendage at the proximal end only, and some have no appendage at all. As the break occurs most frequently as illustrated in fig. 7, however, the appendage may possibly not originate in this way, for it is not quite clear how the formation of a delicate appendage could result from such a wide and blunt connection. Although these irregular forms of spores are often seen, the most usual form is as illustrated in fig. 13. Such

spores are appendaged on the distal end only, are more or less blunt, and show the remains of the original connection at the proximal end. Another very common form is illustrated in fig. 15, similar to fig. 13, except that the spore represented has no appendage, and often possesses a longer and thicker beak.

A knowledge of the catenulate mode of origin of the urediniospores of *Uredinopsis* is of importance, in that it throws light on the natural relationships of the genera of the Uredinaceae (Melampsoraceae). Most authorities (**2, 8**) have grouped the genera of this family in the same general way. For instance, ARTHUR presents four sub-families, namely, the Uredinatae, the Pucciniastratae, the Chrysomyxatae, and the Cronartiatae. In only one of these, the Chrysomyxatae (containing the single genus *Melampsoropsis*), are the urediniospores described as catenulate; in the other three sub-families these spores are described as being "borne singly on pedicels." This classification as defined has already been shown to be inadequate, for investigations made on *Pucciniastrum Agrimoniae* by LUDWIG and REES (**17**) for *Pucciniastrum*, and investigations on *Melampsorella elatina* by MAGNUS (**20**) and LIRO (**16**) for *Melampsorella* have demonstrated the existence of catenulate urediniospores for these forms. While no further work has been published on other species of these genera, it is to be anticipated that they will show the same feature. These two genera were grouped by ARTHUR with *Uredinopsis*, *Hyalopsora*, and other genera in the sub-family Pucciniastratae. LUDWIG and REES pointed out that a readjustment was necessary, and on the basis of the data then at hand they suggested a new classification of this sub-family. They divide the Pucciniastratae into two groups, according to the existence or non-existence of catenulate urediniospores, and include all fern rusts in the non-catenulate group. As my investigations on the urediniospores of *Uredinopsis* indicate that they are catenulate, however, it is evident that their classification demands a second revision.

In order to have some check on my work on the urediniospores of *Uredinopsis*, I have examined the uredinia of *Pucciniastrum pustulatum* on *Epilobium angustifolium* and *E. adenocaulon;* and of *Melampsorella elatina* on *Stellaria graminea* and *Cerastium*

vulgatum. Photographs of sections made from these are shown in figs. 30, 31, 29, and 32 respectively. As explained by LUDWIG and REES, very little can be learned from the arrangement of the mature spores in a pustule; it is only in an immature sorus that the fertile cells or potential spores retain their original positions. If the spores are given off in chains for any particular species, however, this phenomenon is indicated by the arrangement of the producing and developing cells at the base of even an old pustule. Decidedly young stages for any one of these four forms were not at my disposal, but the arrangement of the cells at the bases of their mature fructifications, and the stages in spore formation which were observable in the cells contiguous to the bases, certainly suggested that their urediniospores are produced in chains. On comparing the preparations made from these rusts with those made from the mature pustules of *Uredinopsis*, I found that the chained condition was even more apparent in *Uredinopsis* than in any one of the others.

MARKINGS.—Throughout this study I have had many opportunities of examining the wall of the urediniospores of *Uredinopsis* with reference to their surface markings. The wall of these spores has commonly been described as being characterized by "two opposite longitudinal thickened ridges bearing single rows of minute projections" (2). Occasionally I have observed a certain unevenness of their walls which exhibit the appearance as described, but in the great majority of cases the closest examination of material failed to reveal any such markings. The outside of the wall is seen best in perfectly fresh material examined in a dry mount; when wet the surface always appears to be smooth. In a suitable preparation the surface looks smooth for the main part, but here and there throughout are irregular minute projections, rough patches, and lines. Sometimes, as just stated, these surface markings appear to have a symmetrical arrangement and size, but usually they are irregular in every way.

GERMINATION.—The urediniospores of *Uredinopsis* germinate in a very characteristic manner, as has already been described by FRASER (11), as follows:

> Germ tubes emerged from germ pores, two placed near the beak and two near the base of the spore. The germ tube was that of the usual uredospore

but very small. Two germ tubes only emerged from each spore on germination, usually one from the oppositely placed pores either at the apex or base, but sometimes both on the same side of the spore.

These more usual methods of germination as described by FRASER are illustrated in text figs. 8–10, and text fig. 7 gives the outline of a germinating spore with the two tubes coming from diagonally opposite pores.

TELIOSPORES

The teliospores of *Uredinopsis Osmundae*, *U. mirabilis*, and *U. Phegopteridis* were collected and examined. The principal point of interest brought out was the discovery of their typical mode of distribution within the tissues of their host. The regular position for these spores was found to be just within the epidermis, and not "scattered through the mesophyll" as is usually stated. Occasionally some do occur in the intercellular spaces of the mesophyll, but these are few compared with the large number of spores which form a layer immediately under the epidermis, and most frequently under the lower epidermis. This is strikingly true of *U. Osmundae*, a feature which is shown in figs. 23 and 24, photographs of sections 7 μ in thickness. This layer-like grouping of the teliospores for *U. Osmundae* was observed and reported by MAGNUS (**19**). The same arrangement under the epidermis is illustrated for *U. mirabilis* in fig. 26, and for *U. Phegopteridis* in fig. 22. In both of the latter species they are more widely separated than in *U. Osmundae*, but in all three they are similarly disposed in a layer.

By the time the teliospores are mature the infected portions of the host are usually decidedly withered and not normal, as might be inferred from DIETEL's illustration (**6**, pl. 26, fig. 12). This withered condition is exhibited in figs. 22–26. Likewise the infected patches are yellow and are always limited by the fibrovascular bundles of the leaf (fig. 20); that is to say, the area of a lesion is that of a leaf islet. This limiting of an affected area is shown in fig. 25, in which the leaf veinlet located to the right of the center is seen to separate the withered shrunken tissue on the one side from the normal leaf tissue on the other.

New species

During the progress of this work two new species of *Peridermium* have been found on *Abies balsamea*, and a new form on *Polypodium vulgare*, all of them in the Timagami Forest Reserve. There is good reason to believe that all three are fern rusts of the balsam, and so an account of them properly finds a place in this paper. The last named is a species of *Uredinopsis* (*U. polypodophila*), the first to be reported for the common polypody. Its teliospores have not yet been observed, but the urediniospores were studied carefully, both from fresh material and thin stained microtome sections (fig. 42), and they are so characteristic that this fungus is unhesitatingly referred to the genus *Uredinopsis*. One of the peculiar features of this rust in comparison with other fern rusts is that it apparently fruits only on overwintered fronds; at all events, it has not been found on fronds of the current season.

Of the two species of *Peridermium*, one of them (*P. pycnograndе*) appears to be the alternate stage of the new *Uredinopsis* on *Polypodium vulgare*. Although this relationship has not been established by infection experiments, it is noteworthy that the two (the *Peridermium* on the balsam and the *Uredinopsis* on the common polypody) were always found together. This association was observed in many localities widely separated from one another. Likewise both hosts remained free from infection whenever they were found growing at some distance from one another.

This *Peridermium* is one of the most remarkable that attacks the balsam, not for its size or color, for in both of these features it resembles *P. balsameum*, but for the age of the needles on which it occurs, and its unusually large pycnia. It is always found on old needles, from two to eight years of age, whereas the other known species of *Peridermium* of the balsam, with the single exception of the one pointed out by WEIR and HUBERT (**27**) on the second year needles, are restricted to the new growth. The affected needles turn pale green, but are never blanched as those infected with *P. balsameum*.

The needles which bear the peridermia usually drop from the tree in the course of the following winter. This fact is indicated by the bared stretches of branch axes (figs. 17, 18) found contiguous

to groups of infected needles. In order to establish this point, however, observations were made on certain living balsams in the Timagami Forest Reserve. Trees bearing branches which carried large, closely placed needles heavily infected with this *Peridermium* were chosen during the summer of 1921, and the limits of the infection were carefully marked. These marked branches were observed again in June 1922. By that time most of the needles which carried the infection during the summer of 1921 had dropped off, and later in the summer the needles adjacent to, but just outside the infection limits of the previous season, developed the typical white peridermia and large pycnia. An occasional needle which carried the infection of 1921 remained on the branch and developed peridermia again in 1922, in which case the blackened scars left by the 1921 peridermia could always be seen on the under surface of the leaf. Very commonly, too, the infected portions are associated with a peculiar multiplication of twigs, producing a loose broom effect (figs. 16, 19). These brooms are not found on a newly infected portion, but develop from year to year with the progress of the rust. This abnormal increase of branches never produces a dense mass of twigs, as in the case of *Melampsorella elatina*, but all the characters of a thicker broom are exhibited, although on a smaller scale. There is also a change in the normal direction of growth (figs. 16, 19). Fig. 19 shows the final loose broomlike effect.

The bark from infected branches was examined and fungus threads were found growing among the cells of the cortex. As yet, however, no work has been done to prove that the mycelium observed belonged to the same organism which produced the peridermia on the leaf, but the presence of fungus threads among the cells of the cortex, associated with the spread of the fungus from year to year up and down the branch, and the stimulation of the tree to abnormal growth, suggest that this *Peridermium* is perennial on *Abies balsamea*.

The second *Peridermium* (*P. pycnoconspicuum*) appears to be the alternate stage of *Hyalopsora Aspidiotus* (Peck) Magnus. The balsams affected with it were found constantly associated with *Phegopteris Dryopteris* bearing a copious growth of *H. Aspidiotus*. To test this relationship experimentally, preliminary tests were

made as follows. On June 24, 1922, four boxes of *Phegopteris Dryopteris* free from disease, two plants in each box, were removed to the laboratory from a locality free from *Hyalopsora* and *Uredinopsis*, and which remained free for the rest of the summer. The ferns in three of the boxes were inoculated with the yellow aeciospores of this second new *Peridermium*, and were then left in a moist chamber for two days. They were then placed out of doors in a location far removed from other plants of *Phegopteris Dryopteris*, and at least two miles from any known infection of the *Hyalopsora*. The ferns in the fourth box were kept as controls. On July 12 pustules giving off the typical golden-yellow spores of *Hyalopsora* had developed on both plants in one of the boxes. A few days later one of the plants in a second box showed a similar infection; thus three out of the six inoculated ferns were infected with *H. Aspidiotus*, which may be considered a fair showing in view of the advanced age of the aecia from which the inoculum was taken, and some days of dry weather to which these delicate ferns were subjected. The controls remained free from infection. While these experiments cannot be considered as final, the results obtained from them and the phenomena of association in the field are strongly indicative of the suggested relationship.

There have been many speculations regarding the aecial stage of *Hyalopsora*. It has always been regarded as a peculiar genus, and its life history has been imperfectly understood and variously explained. Several investigators have assumed that the aecial stage of *Hyalopsora* might be found on a conifer, and some inoculation experiments have been made by them, although without success. BARTHOLOMEW (**4**), after studying this rust on the ferns, was convinced that its alternate stage would be found on conifers. Several years previous BUBÁK (**5**) had made cross inoculations on *Abies* and *Pinus*. DIETEL (**7**) in 1911 made further tests, and according to P. and H. SYDOW (**26**), BUBÁK and KLEBAHN inoculated, but without success, species of *Abies*, *Picea*, *Larix*, and *Pinus* with telia of *H. Aspidiotus*. WEIR and HUBERT (**28**) report that they made similar unsuccessful inoculations on species of *Abies*, *Tsuga*, and *Pteris* (*Pteridium*). Incidentally it may be noted that the last named investigators carried out tests that would indicate

that the urediniospores of *H. Aspidiotus* and *H. Polypodii* may winter over. Further, in none of the studies on this genus has there been any report of inoculations in the other direction, that is, from a *Peridermium* to the fern host. Thus the experiment reported in this paper is the first evidence obtained by means of inoculation that *Hyalopsora* is a heteroecious rust, and that its aecial stage actually exists on a conifer.

This *Peridermium* has also proved to be interesting in comparison with the other species of *Peridermium* found on *Abies balsamea*. It is one of the earliest to appear. It is shorter than most others, and characterized by an early apical dehiscence of bright yellow spores. The affected needles are pale greenish and rather sparsely scattered. One of the most striking things observed was the fact that the rust appears to be restricted to the needles of the third previous year. Thus of twenty-nine specimens taken at random in June 1922, the rust was found in every instance on the needles of 1920 only, but perhaps the most important feature of all is the occurrence of conspicuous yellowish pycnia of very large size.

The discovery of such unusual pycnia lends added interest to a comparative study of the pycnia of the rusts found on the balsam. A complete survey of this subject would involve an examination of the pycnia of various species of *Pucciniastrum*, *Melampsora*, *Melampsorella*, *Hyalopsora*, and *Uredinopsis*. Incidentally it may be in place here to report that occasional pycnia were found associated with the peridermia of *Calyptospora columnaris;* but even the studies reported herewith indicate that the pycnia are very important diagnostic structures. To demonstrate this it will be sufficient to call attention to the pycnia of four species of *Peridermium* on *Abies balsamea*, namely, *P. balsameum* (*Uredinopsis* on the fern), *P. pycnogrande* (probably *U. polypodophila* on *Polypodium vulgare*), *P. pycnoconspicuum* (probably *Hyalopsora Aspidiotus* on *Phegopteris Dryopteris*), and *Pucciniastrum pustulatum* (telial stage on *Epilobium angustifolium*). Taking these in order, the distinguishing characters of their pycnia can be summarized as follows.

The pycnia of *Peridermium balsameum* (figs. 33, 34) are irregularly scattered; inconspicuous on the exterior; when mature extended both above and below the epidermis; in section

hemispherical to spherical; comparatively small, 100–130 μ broad by 30–50 μ high.

The pycnia of *P. pycnograndе* (figs. 38, 39) are alternated regularly with the peridermia; inconspicuous on the exterior; subepidermal; deep seated in the mesophyll; in section conspicuous and spherical; large, 180–250 μ in diameter.

The pycnia of *P. pycnoconspicuum* (figs. 35–37) are alternated either regularly or irregularly with the peridermia, and sometimes in rows without any peridermia; conspicuous as round yellow spots on the exterior; extended well past the epidermis into the mesophyll; in section conspicuous and oval in outline; large, 400–500 μ long by 90–120 μ deep.

The pycnia of *Pucciniastrum pustulatum* (figs. 40, 41) are irregularly scattered; easily seen with a hand lens, but not conspicuous on the exterior; subcuticular and superficial, the epidermal cells being only partially occupied; in section flattened; small, 50–110 μ broad by 20–30 μ high.

The type specimens of the new species are deposited in the herbarium of the Department of Botany of the University of Toronto. The detailed description of them follows.

Peridermium pycnograndе, n. sp.—O. Pycnia hypophyllous, alternating with aecia in two rows, one on each side of midrib, forming distinct but not conspicuous light spots on the under surface. In section of leaf conspicuous, subepidermal, deep seated in the mesophyll, oblong or spherical, 180–250μ in diameter.

I. Aecia from mycelium freely distributed through mesophyll, hypophyllous, in two rows, one on each side of midrib, cylindrical, 0.25–0.3 mm. in diameter, and often 1 mm. high; peridermium colorless, delicate, rupturing at apex, becoming irregularly lacerate, cells elongated, 17–25×32–47 μ, walls thick, 2.5–3.4 μ, verrucose, overlapping; aeciospores globoid or broadly ellipsoid, 18–24×22–30 μ, wall colorless, rather thin, 0.8–1.3 μ, verrucose.

On *Abies balsamea* (L.) Mill. on two to eight-year old leaves, found near *Polypodium vulgare* around Lake Timagami, Ontario, Canada, appearing during July, August, and early September.

Peridermium pycnograndе, sp. nov.—O. Pycnidiis hypophyllis cum aecidiis alternis in duo series utrimque ad nervum dispositis

in maculis pallidis, distinctis sed non conspicuis, paginae inferioris; secto folio conspicuis, subepidermicis, penitus in mesophyllo insidentibus, oblongis v. sphaericis, 180–250 μ diam.

I. Aecidiis e mycelio orientibus copiose per mesophyllum distributo, hypophyllis, in duo series utrimque ad nervum dispositis, cylindratis, 0.25–0.3 mm. diam., saepe 1 mm. alt. Peridio hyalino delicato, apice erumpente, deinde irregulariter lacerato, cellulis elongatis, 17–25×32–47 μ, parietibus crassis, 2.5-3 μ, verrucosis imbruatis; aecidiosporis globoedis v. late ellipsoideis, 18–24×22–30 μ, tunica hyalina, subtenui, 0.8–1.5 μ, verrucosa.

Hab. in 2–8 annorum foliis *Abietis balsameae* (L.) Mill. prope *Polypodium vulgare* circum Lacum Timagami, Ontario, Canada, mensibus Julio, Augusto, et Septembri.

Uredinopsis polypodophila, n. sp.—II. Uredinia hypophyllous, few, minute, pale yellowish brown, 0.2–0.4 mm. across; peridium irregularly dehiscent, spores exuded in white filiform mass; urediniospores oval or fusiform, 45–55×15–23 μ, acute or acuminate above, with an apex often prolonged into a moderately strong beak 5–12 μ long; wall colorless, thin, 1 μ, smooth.

III. Not observed.

On *Polypodium vulgare* L., on fronds of the previous summer, found in shade of young balsam trees around Lake Timagami, Ontario, Canada, appearing during July, August, and early September.

Uredinopsis polypodophila, sp. nov.—II. Uredosoris hypophyllis, paucis, minutis, pallide flavobrunneis, 0.2–0.4 mm. diam., peridermio irregulariter dehiscente; sporis in molem albam filiformem exeuntibus; urediniosporis ovoideis vel fusiformibus, 45–55×15–23 μ, in super acutis vel acuminatis, apice saepe in rostrum moderate firmum 5–13 μ longum producto, tunica hyalina, tenui, 1 μ levi.

III. Teleutosporis ignotis.

Hab. in foliis anni praeteriti *Polypodii vulgaris* L. sub umbra arborum juniorum *Abietis balsamae*, circum Lacum Timagami, Ontario, Canada, mensibus Julio, Augusto, et Septembri.

Peridermium pycnoconspicuum, n. sp.—O. Pycnia hypophyllous, alternating irregularly with aecia in two rows, one on each side of midrib, forming conspicuous round yellow spots on the under

surface. In section of leaf conspicuous, oval, subepidermal, 400–500 μ long by 90–120 μ deep.

I. Aecia from mycelium freely distributed through mesophyll, hypophyllous in two rows, one on each side of midrib, bladder-like, spherical, low, 0.4–0.5 mm. in diameter and often only 0.2 mm. high; peridium colorless, delicate, rupturing at apex, cells oblong, 15–20×18–32 μ, walls thick, 2–3 μ; aeciospores globoid or broadly ellipsoid, 13–18×15–25 μ, yellow; wall colorless, thin, verrucose.

On *Abies balsamea* (L.) Mill. on leaves of the third previous season; that is, the collections of 1922 occurred on the needles of 1920. Found near *Phegopteris Dryopteris* affected with *Hyalopsora Aspidiotus* around Lake Timagami, Ontario, Canada, appearing during June.

Peridermium pycnoconspicuum, sp. nov.—O. Pycnidiis hypophyllis cum aecidiis in duas series irregulariter alternantibus utrimque ad nervum dispositis in maculis orbiculatis fulvis conspicuis paginae inferioris; secto folio conspicuis, ovatis subepidermicis, 400–500 μ long, 90×120 μ alt.

I. Aecidiis e mycelio copiose per mesophyllum distributo, hypophyllis in duas series utrimque ad nervum dispositis, vesicae similibus, sphericis, depressis, 0.4–0.5 mm. diam., et saepe 0.2 mm. modo alt; peridio hyalino, delicato, apice erumpente, cellulis oblongis, 15–20×18–32 μ, parietibus crassis, 2–3 μ; aeciosporis globoedis vel late ellipsoideis, 13–18×15–25 μ, clarissime; fulvis tunica hyalina, tenui, verrucosa.

Hab. in tertii anni antecedentis foliis *Abietis balsameae* (L.) Mill. i.e. anni 1922 collationes in foliis anni 1920 inventae sunt. Prope *Phegopterim Dryopterim Hyalopsora Aspidiota* affectis circum Lacum Timagami, Ontario, Canada, mense Junio.

Summary

1. *Uredinopsis*, originally defined as a genus of imperfect fungi parasitic on ferns, was proved to be a genus of rusts by DIETEL in 1895. Heteroecism in *Uredinopsis* was first demonstrated by FRASER in 1913, when by means of inoculation experiments he established the relationship of some of the species to *Peridermium balsameum*.

2. The genus is characterized by four recognized types of spores: aeciospores, pycnospores, urediniospores, and teliospores. In

addition, some of the earlier investigators described a more or less irregularly occurring form on the fern host, interpreted variously as resting urediniospores, one-celled teliospores, and aeciospores. Studies recorded in this paper suggest that in some cases at least the urediniospores of a *Hyalopsora* have been misinterpreted as spores of the *Uredinopsis*. This type has never been found for any species studied, and a wide range of material was examined.

3. The urediniospores of *Uredinopsis*, unique because of their large size, fusiform shape, terminal appendages, and their emergence in spore horns, have heretofore been described as being borne singly. They are now shown to be produced in chains. Thus *Uredinopsis* should be grouped among those genera which are characterized by catenulate urediniospores.

4. No distinct evidence of constant markings on the urediniospores could be established.

5. FRASER'S account of the germination of the urediniospores is confirmed.

6. Teliospores of *Uredinopsis Osmundae*, *U. mirabilis*, and *U. Phegopteridis* have been studied carefully, and are found to occur in a more or a less regular layer immediately under the epidermis, and not irregularly scattered through the mesophyll, as commonly stated.

7. Three new species are described, namely, *Peridermium pycnograndе* and *P. pycnoconspicuum*, both on *Abies balsamea*, and *Uredinopsis polypodophila* on *Polypodium vulgare*.

8. Balsams affected with *Peridermium pycnograndе* were found commonly associated with the common polypody affected with *Uredinopsis polypodophila*, and field evidence indicates a connection between the two. The peridermia of this species are white throughout, and are accompanied by pycnia of very large size. This rust is also remarkable in that it occurs on needles from two to eight years of age and never on needles of the current season. This fungus is responsible for the growth of loose brooms in which it appears to be perennial.

9. Balsams affected with *Peridermium pycnoconspicuum* were found constantly associated with the oak fern *Phegopteris Dryopteris* affected with the uredinial stage of the rust *Hyalopsora Aspidiotus*

(Peck) Magnus. Field associations and preliminary culture experiments indicate that this peridermium is the aecial stage of *Hyalopsora Aspidiotus*. This is the first evidence indicating the heteroecism of any species of *Hyalopsora*. The peridermia of *Peridermium pycnoconspicuum* are columnar, the aeciospores are yellow, and the pycnia are large and conspicuous. The rust was found only on needles of the third previous season; that is, the collections of 1922 occurred on the still adhering needles of 1920.

10. The pycnia of *Peridermium balsameum*, *P. pycnogrande*, *P. pycnoconspicuum*, and *Pucciniastrum pustulatum* are here described and compared photographically; and they are shown to be distinctly characteristic for each species, and hence are important diagnostic features.

This work was done under the direction of Professor J. H. FAULL, University of Toronto, to whom I am indebted, not only for material and certain illustrations, but also for advice and assistance at every stage of this work. The investigations conducted during 1919 were made possible by a studentship awarded by the Honorary Advisory Council for Scientific and Industrial Research for Canada; and the summer work of 1921 and 1922 by a grant from the Special Research Fund of the University of Toronto. I wish to express my obligations to both the institutions from which this assistance was received. I am also under obligations to the Ontario Forestry Branch of the Department of Lands and Forests for the facilities extended in their laboratory in the Timagami Forest Reserve. For the translation of my English descriptions into Latin I am indebted to Miss MARY NEEDLER.

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EXPLANATION OF PLATES I–V

FIG. 1.—Camera lucida drawing of immature uredinium of *Uredinopsis Phegopteridis* on *Phegopteris Dryopteris;* ×953.

FIG. 2.—Composite drawing of mature uredinium of *Uredinopsis Phegopteridis* on *Phegopteris Dryopteris;* ×282.

FIGS. 3–11.—Stages in development of urediniospore of *Uredinopsis.*

FIG. 3.—Young spore in first stage of development amongst mycelium at base of sorus; ×827.

FIG. 4.—Spore as in fig. 3, after one division; ×827.

FIG. 5.—Later stage; there has been a second division in lower spore; cross wall is being formed; ×827.

FIG. 6.—Condition following that illustrated in fig. 5; small round cells starting to elongate; ×827.

FIG. 7.—Two cells of spore chain; distal cell is almost a mature spore (common form of development); ×827.

FIG. 8.—Protoplasm of two spores just starting to separate, leaving cell wall free; ×827.

FIGS. 9–11.—Spore chains illustrating more shrinkage of protoplasm than shown in fig. 8; ×827.

FIGS. 12–15.—Typical urediniospores of *Uredinopsis* all arranged with apex uppermost; fig. 13 illustrates commonest form; ×827.

FIG. 16.—Typical and fully developed broom on *Abies balsamea* infected with *Peridermium pycnogrande* (photographed by Dr. J. H. FAULL).

FIG. 17.—Branch of *Abies balsamea* infected with *Peridemium pycnogrande;* considerable defoliation taken place; most of needles dotted with white peridermia.

FIG. 18—Branch of *Abies balsamea* infected with *Peridermium pycnogrande;* peridermia show clearly; also loss of needles and abnormal development of twigs is just starting (photographed by Dr. J. H. FAULL).

FIG. 19.—*Abies balsamea* badly infected with *Peridermium pycnogrande,* and exhibiting abnormal multiplication of branches at various places (photographed by Dr. J. H. FAULL).

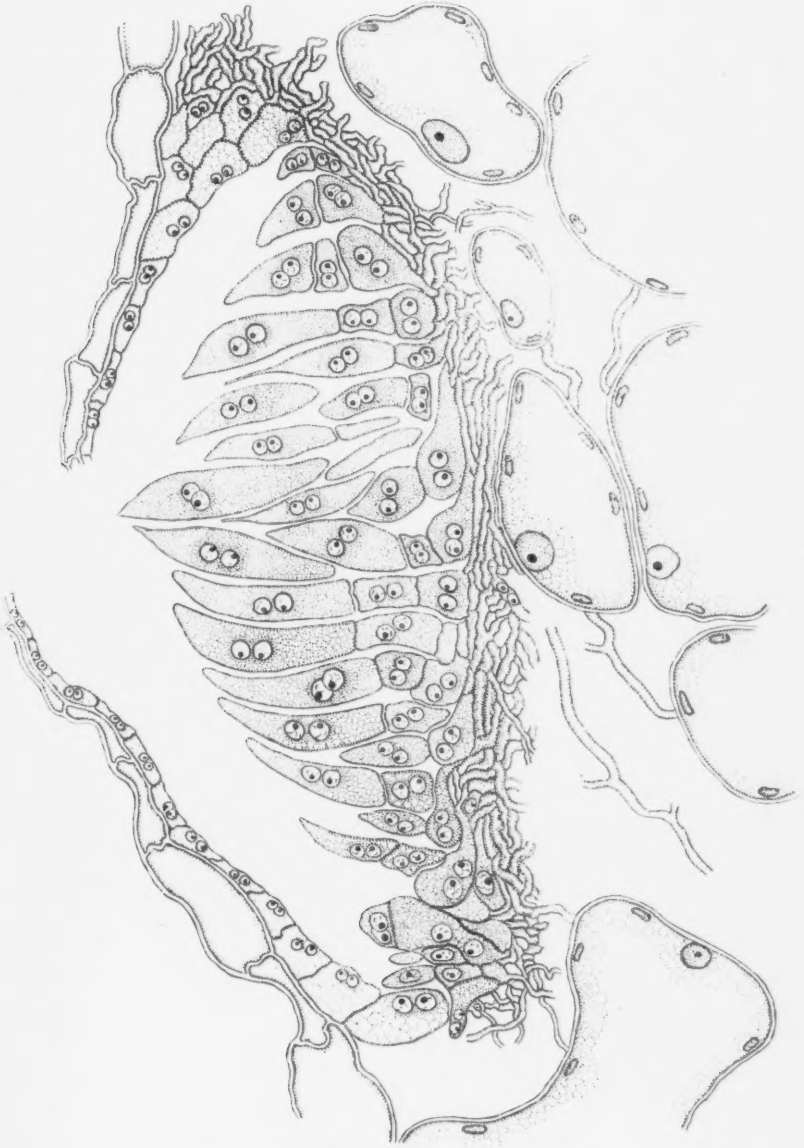
FIG. 20.—Pinna of *Osmunda Claytoniana* infected with *Uredinopsis Osmundae;* infected leaf islets are lighter color (photographed by Dr. J. H. FAULL).

FIG. 21.—Uredinium of *Hyalopsora Aspidiotus,* typical unbroken pustule so often found; break visible in covering layer of epidermis caused in cutting; ×200.

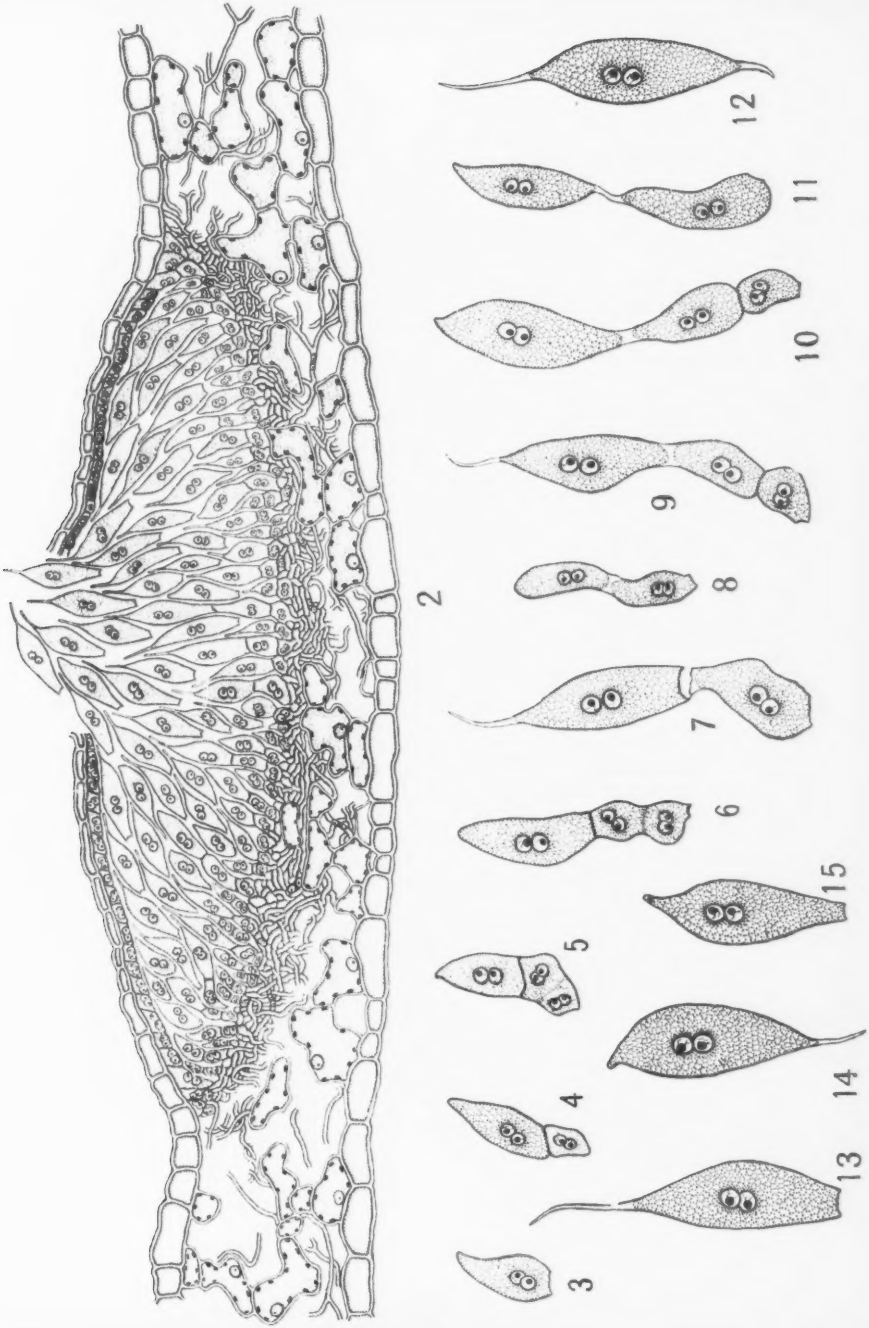
FIG. 22.—Teliospores of *Uredinopsis Phegopteridis* on *Phegopteris Dryopteris;* ×300.

FIG. 23.—Teliospores of *Uredinopsis Osmundae* on *Osmunda Claytoniana;* ×144.

FIG. 24.—Teliospores of *Uredinopsis Osmundae* on *Osmunda Claytoniana;* central part of section shown in fig. 36; ×300.



BELL on RUSTS OF ABIES



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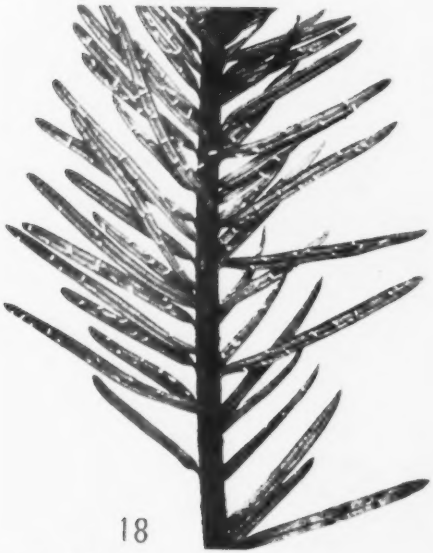

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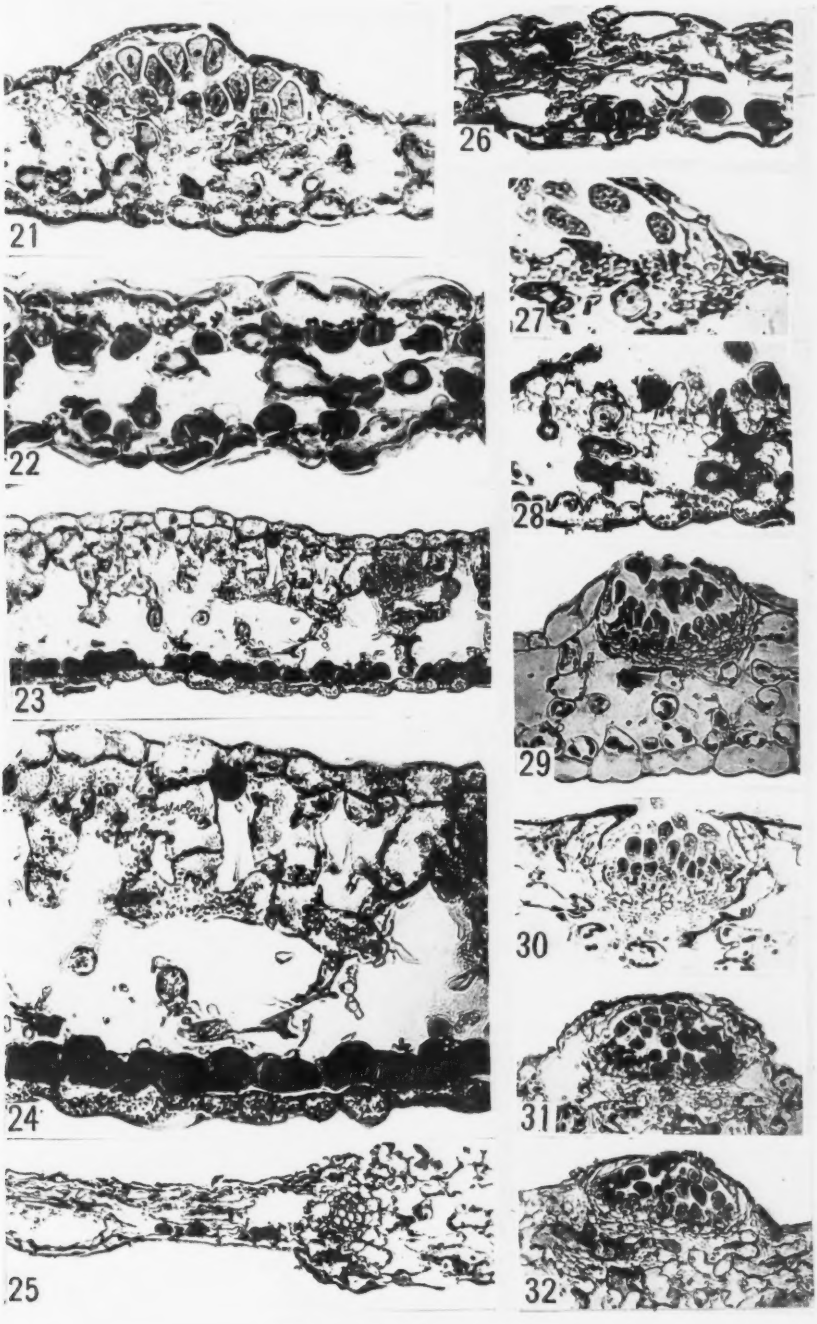
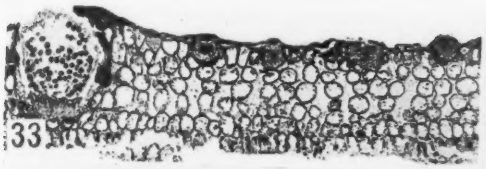
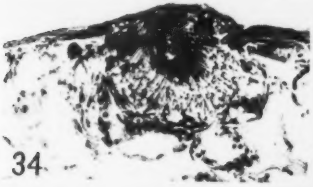
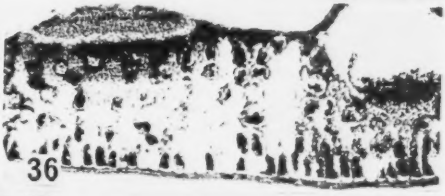
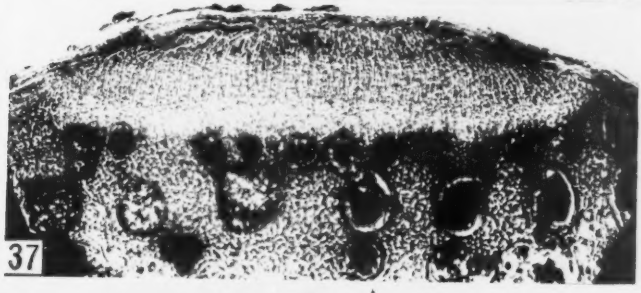


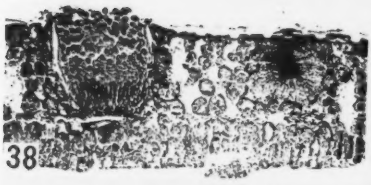
BELL on RUSTS OF ABIES

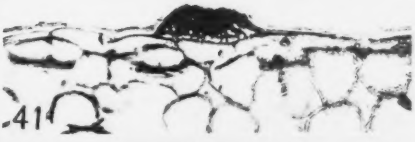


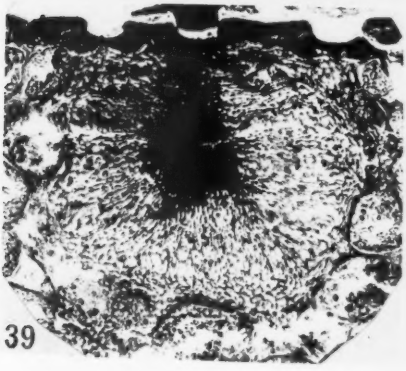


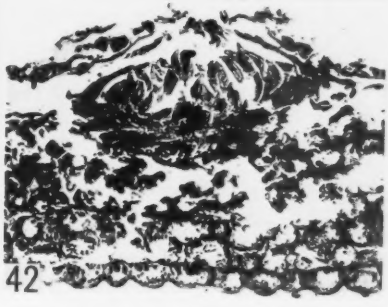












BELL on RUSTS OF ABIES

FIG. 25.—Section of frond of *Onoclea sensibilis* infected with *Uredinopsis mirabilis;* infected and normal portions of leaf separated by fibrovascular bundle; infected portion withered and shrunken, showing four teliospores immediately under epidermis; ×144.

FIG. 26.—Teliospores of *Uredinopsis mirabilis* on *Onoclea sensibilis;* tissue of host decidedly withered; ×300.

FIG. 27.—Uredinium of *Hyalopsora Aspidiotus* (note that a spore is borne on inner surface of basal mycelial mat); ×200.

FIG. 28.—Uredinium of *Hyalopsora Aspidiotus:* at left hand end of pustule there is a spore borne in center of basal mycelial mat; ×200.

FIG. 29.—Uredinium of *Melampsorella elatina* on *Stellaria graminea;* ×200.

FIG. 30.—Uredinium of *Melampsorella elatina* on *Cerastium vulgatum;* ×200.

FIG. 31.—Uredinium of *Pucciniastrum pustulatum* on *Epilobium angustifolium;* ×200.

FIG. 32.—Uredinium of *Pucciniastrum pustulatum* on *Epilobium adenocaulon;* ×200.

FIG. 33.—Longitudinal section of leaf of *Abies balsamea,* showing aecium and pycnia of *Peridermium balsameum;* ×48.

FIG. 34.—Pycnium of *Peridermium balsameum;* ×200.

FIG. 35.—Longitudinal section of leaf of *Abies balsamea,* showing aecium and pycnium of *Peridermium pycnoconspicuum;* ×46.

FIG. 36.—Same as fig. 35; ×92.

FIG. 37.—Pycnium of *Peridermium pycnoconspicuum;* higher magnification of pycnium shown in fig. 36; ×200.

FIG. 38.—Longitudinal section of leaf of *Abies balsamea,* showing section and pycnium of *Peridermium pycnogrande;* ×46.

FIG. 39.—Pycnium of *Peridermium pycnogrande;* higher magnification of pycnium shown in fig. 51; ×200.

FIG. 40.—Longitudinal section of leaf of *Abies balsamea,* showing aecium and pycnia of *Pucciniastrum pustulatum;* ×46.

FIG. 41.—Pycnium of *Pucciniastrum pustulatum;* ×200.

FIG. 42.—Uredinium of *Uredinopsis polypodophila;* ×200.

NOTES ON NEOTROPICAL ANT-PLANTS

III. CORDIA NODOSA LAM.

I. W. BAILEY

(WITH PLATES VI, VII, AND FIVE FIGURES)

Introduction

There are numerous tropical plants that have curious nodal or internodal swellings which are inhabited by ants, such as species of *Myristica*, *Kibara*, *Anthobembix*, *Pleurothyrium*, *Humboldtia*, *Schotia*, *Platymiscium*, *Chisocheton*, *Aphanamixis*, *Endospermum*, *Barteria*, *Gertrudia*, *Maieta*, *Epitaberna*, *Cordia*, *Clerodendron*, *Nauclea*, *Uncaria*, *Randia*, *Plectronia*, *Cuviera*, *Sarcocephalus*, etc. Most of these myrmecodomatia are simple cauline hypertrophies, but certain of the neotropical species of *Cordia* have very complex structures, which are so remarkable morphologically as to justify discussing them at considerable length in the following pages.

Morphology

Two sections of the Borraginaceous genus *Cordia* are provided with myrmecodomatia, Physoclada and Gerascanthus. The most classical representative of these ant-plants is one of the Physocladae which is commonly referred to *Cordia nodosa*. SCHIMPER (**16**) encountered the plant in Pernambuco and studied its morphological peculiarities.

The large leaves which, like the stem, are provided with long reddish hairs, are alternate, paired or grouped in false verticils of four. The subnodal portion of the stem, subtending each verticil of leaves, is strongly thickened and angular, and usually (although not invariably) is provided with a long bladder-like swelling. This pouch is jacketed internally as well as externally by a cuticularized epidermis and numerous trichomes. It subtends the lowest of the four leaves, in the axil of which is a small apical outlet which is not excavated by the ants. Above this leaf the thickened and much

compressed primary axis gives rise to lateral inflorescences which are inserted in the axils of the three remaining leaves.

SCHIMPER concluded that the myrmecodomatium is formed by the lateral enlargement of the base of the petiole of the lowest leaf, which is adnate to the compressed portion of the main axis. SCHUMANN (17) questioned the accuracy of SCHIMPER'S observations. He argued from analogies with supposedly similar domatia of *Duroia hirsuta* and of various Gerascanthi, that the inflorescences are terminal, and that the bladder-like swellings are cauline, rather than adnate foliar structures. MEZ (14) agreed with SCHUMANN that the inflorescences are terminal, but he inferred that the domatia are formed by lateral invaginations. His description of the morphology of the myrmecodomatia may be summarized as follows:

> The hypertrophied portion of the main axis bears two approximately opposite leaves, and terminates in an inflorescence. The lower leaf of this pair is subtended by the bladder-like swellings, and the axis of vegetative elongation, which is of secondary order, arises from its axil. The two remaining leaves of the false verticil are the first leaves of this secondary axis. Externally the bladder-like structure is delimited by two longitudinal grooves, which extend from the base of the myrmecodomatium to its apex. Serial transverse sections of the domatium reveal the following sequence of changes. Below the hypertrophy the stem is of normal structure. In passing upward through the transitional region, one of the grooves remains unmodified, whereas the other deepens, producing an embayment in the contour of the vascular cylinder and medulla. The pith splits, and extensions of the fibrovascular and cortical tissues invaginate or bend inward around the medullary cavity. The invagination continues until the cortical and fibrovascular tissues intersect and unite with homologous tissues on the opposite side of the stem. Thus the pith is divided and compressed into two narrow, curved strands, each of which is surrounded on all sides by fibrovascular and cortical tissues, and a commodious internal cavity is concomitantly formed which is jacketed by a cuticularized epidermis and numerous trichomes.

MEZ concluded that these invaginations were originated by minute insects which lived in the lateral grooves, and that they subsequently became accentuated and inherited as adaptations for housing ants. During the phylogenetic development of the myrmecodomatia, the margins of the longitudinal cleft, formed by the lateral invagination, fused together and isolated the internal layer of epidermal tissue. The position of this suture is indicated by a gap in the outer ring of sclerenchymatous tissue.

One of the commonest myrmecophytes of the Kartabo region of British Guiana is a *Cordia* which resembles the plant that SCHIMPER investigated in Pernambuco. Its dichotomously branching, hirsute cauline axes periodically form subnodal hypertrophies which subtend false verticils of four large hairy leaves and small inflorescences. These myrmecodomatia are jacketed both internally and externally by a cuticularized epidermis and numerous trichomes (except where they have been trimmed away by the ants), and are provided with preformed apical apertures. They are more or less elongate-turbinate, and differ from those described and figured by SCHIMPER in having no large, conspicuous, lateral, bladder-like swelling or pouch (text fig. 1). The four leaves of the false verticil are inserted at slightly different levels. The lowest leaf subtends a small axillary bud, which may occasionally develop into a lateral inflorescence. The leaf on the opposite side of the domatium is the highest of the four, and subtends the entrance aperture. The leaves of the remaining pair are inserted at right angles to this pair, and their axillary buds commonly give rise to dichotomous, lateral vegetative axes. Above the level of the leaves the main axis terminates in an inflorescence.

Serial transverse sections of the lower two-thirds of the myrmecodomatia show no indication of a putative flattened stem and adnate petiole, nor indeed of a lateral invagination (figs. 1, 4). The more or less circular wall is composed of concentric rings of highly differentiated tissues. Under higher magnification (figs. 5, 6) the sequence of tissues is seen to be: (1) outer epidermis, (2) cortical parenchyma, (3) sclerenchyma, (4) phloem, (5) xylem, (6) medullary parenchyma, (7) xylem, (8) phloem, (9) sclerenchyma, (10) cortical parenchyma, (11) inner epidermis. There are no connections between the rings of homologous tissue. The inner fibrovascular cylinder, whose tissues have an inverse orientation, is more or less rudimentary, and the increase in thickness of the wall of the domatium is due to cambial activity in the centrifugal or outer fibrovascular cylinder (cf. figs. 1 and 4). In passing upward toward the verticil of leaves, four arcs of the centrifugal cylinder become accentuated and project outward (fig. 9, text fig. 2). The longest arc of the four, which is a laterally enlarged basal projec-

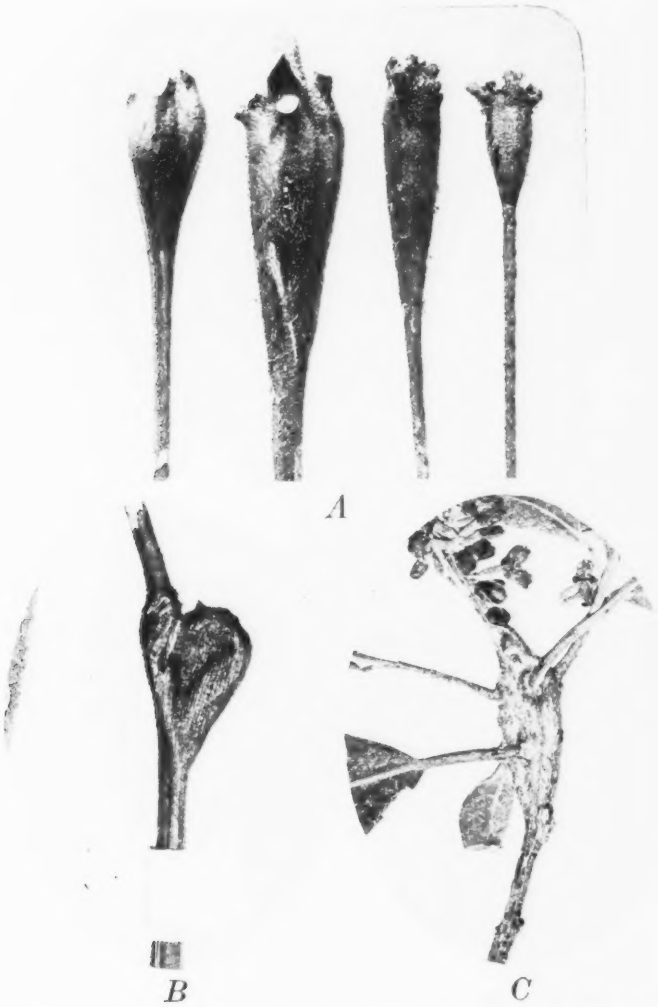


FIG. 1.—*A*, myrmecodomatia of *Cordia nodosa*; *B*, myrmecodomatium of *Cordia hispidissima* (Burchell no. 9637); *C*, myrmecodomatium of *Cordia Gerascanthus* Jacq. (Herbarium J. D. Smith no. 4365).

tion of the trace of the highest leaf, tends to split off from the fibrovascular cylinder before the others. As it does so, the centripetal fibrovascular cylinder divides into two portions, one of which becomes attached to the centrifugal cylinder and the other to the departing leaf trace. The former portion becomes an integral part of the centrifugal cylinder, whereas the latter portion gradually dies out at a higher level, leaving a normal arc-shaped trace which passes into the petiole of leaf (text fig. 3*A*). As indicated in this diagram, the entrance aperture is located in the axil of this leaf.

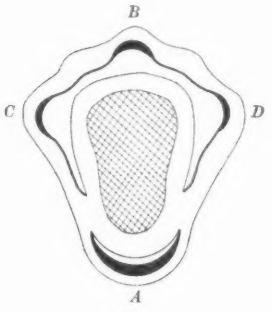


FIG. 2

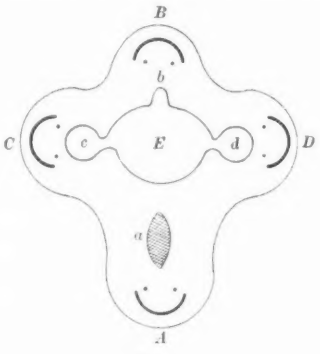


FIG. 3

FIGS. 2, 3.—Fig. 2, Transverse section of apical portion of myrmecodomatium of *Cordia nodosa*, showing traces of leaves *A*, *B*, *C*, and *D*.; fig. 3, Transverse section of apical portion of myrmecodomatium of *Cordia nodosa*, cut at somewhat higher level than that shown in fig. 2: *a*, entrance aperture in axil of leaf (*A*); *c*, *d*, axes of vegetative shoots in axils of leaves (*C*) and (*D*); *b*, rudimentary bud in axil of leaf (*B*); *E*, axis of terminal inflorescence.

The opposite leaf (*B*) subtends a dormant bud (*b*). Leaves *C* and *D* subtend the lateral vegetative shoots *c* and *d*, and the main axis (*F*) terminates in an inflorescence.

What then is the morphological significance of these extraordinary structures? The hypotheses of SCHIMPER, SCHUMANN, and MEZ do not afford an adequate explanation of all phases of their ontogenetic or phylogenetic development. Below the subnodal hypertrophy, the stem is of normal structure. Above this level it rapidly increases in girth. As it does so, the circumference of its concentric layers of epidermal, cortical, and fibrovascular

tissues becomes correspondingly enlarged, and a commodious internal cavity is concomitantly formed in the dilated core of medullary parenchyma. This chamber, unlike that of the caulinary domatium of other myrmecophytes, is characterized by being jacketed by centripetal layers of epidermal, cortical, and more or less rudimentary fibrovascular tissues. In other words, there is no indication of a compressed stem and adnate petiolar enlargement, nor of an extensive lateral invagination. The centripetal layers of epidermal, cortical, and fibrovascular tissues unite with the homologous centrifugal layers only in the apical portion of the wall of the domatium which surrounds the small circular aperture.

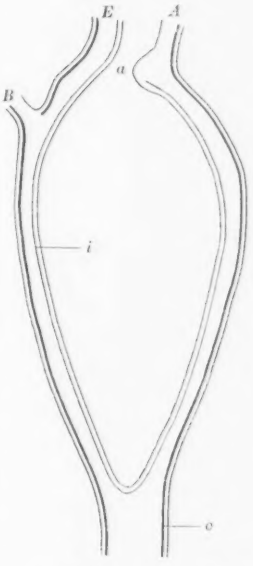


FIG. 4.—Longitudinal section of myrmecodomatium of *Cordia nodosa*, showing centrifugal (*o*) and centripetal (*i*) fibrovascular tissues: *a*, entrance aperture in axil of leaf (*A*); *E*, terminal inflorescence.

A detailed study of the morphology of the myrmecodomatia, during successive stages of their ontogeny, indicates that they are hypertrophied portions of the cauline axes, whose medullary cavities are jacketed by layers of invaginating tissues. The invagination does not originate, however, in a longitudinal lateral groove, but in the axil of one of the leaves of the pseudo-apical verticil. As it develops, it produces an elongate-saccate ingrowth of the epidermal, cortical, and fibrovascular tissues into the rapidly enlarging core of medullary parenchyma (text fig. 4). The absence of even a rudimentary bud in the axil of a leaf which subtends an entrance aperture suggests that this growing point may be concerned in the formation of the invagination. If it is, the invagination may be visualized as the homologue of an ingrowing lateral shoot, and its formation may be likened to what happens when one finger of a glove is retracted so that it ultimately projects inward instead of

outward. In exceptional cases, invaginations may develop in the axils of two of the leaves of a single verticil. Fig. 8 illustrates a transverse section of an abnormal, compound domatium of this type.

It is of interest to compare these myrmecodomatia with those of other representatives of the Physocladae which have conspicuous, lateral, bladder-like swellings. Burchell's no. 9637, which is referred to *Cordia hispidissima* DC., and the Rusby Expedition's nos. 778 and 1035 have myrmecodomatia of this type. Serial transverse sections of these hypertrophies reveal the following histological details. Below the domatium the stem is of normal structure, but above this level it becomes constricted by two longitudinal furrows, and the pith appears bilobed. An internal cavity is formed in one of these lobes, which is jacketed by layers of inversely oriented epidermal, cortical, and fibrovascular tissues (fig. 7). The arc of the outer or centrifugal fibrovascular cylinder which surrounds this lobe becomes detached. As it does so, the inner or centripetal fibrovascular ring splits into two arcs, each of which attaches itself to one of the two outer arcs of homologous tissue (fig. 2). As the domatium enlarges, one lobe-shaped portion of the axis, the one which partially surrounds the internal cavity, enlarges more rapidly than the other and forms the bladder-like, lateral hypertrophy (cf. figs. 2 and 7). In the apical portion of the domatium, this lobe contracts, the inner arc of fibrovascular tissue fades away, and the outer arc passes into the petiole of the leaf which subtends the entrance aperture. The outer arc of fibrovascular tissue in the opposite lobe gives off three leaf traces, and in combination with the inner arc, subsequently forms the steles of four cauline axes, three lateral and one terminal (text fig. 3). The terminal axis forms the inflorescence. One or more of the lateral axes elongate to form vegetative shoots, or in certain cases lateral inflorescences.

It is evident that the myrmecodomatia of these plants are of the same fundamental morphological type as those which the writer studied in British Guiana. The striking difference in their external form is due to the fact that in the case of the former plants the subnodal enlargement of the stem is unilateral, and the basal projection of the trace of the leaf which subtends the entrance aperture is set off from the central cylinder at a much lower level.

Such structural differences are of not uncommon occurrence in closely related species of dicotyledons. Thus the basal projections of the leaf traces in one species or variety may be differentiated or even set off from the stele at a much lower level than they are in others. In view of these facts, the following conclusions concerning the morphological peculiarities of the Physocladae seem justified: (1) the myrmecodomatium is formed by an invagination of epidermal, cortical, and fibrovascular tissues which originates in the axil of one of the leaves of the false verticil, and which develops into the interior of a more or less symmetrical or unilateral, subnodal enlargement of the cauline axis; (2) this cauline axis usually terminates in an inflorescence; (3) the buds in the axils of three of the leaves of the false verticil of four give rise to one or more lateral axes of vegetative elongation, but in certain cases may form one or more lateral inflorescences.

The next question is, are the myrmecodomatia of the Gerascanthi homologous morphological structures? Although the writer has not encountered these myrmecophytes in the field, he has studied dried specimens of their myrmecodomatia in the extensive collections of the National Herbarium, the New York Botanical Garden, and the Gray Herbarium. The domatia vary greatly in size, shape, and distribution in different representatives of even a single species. When present, they are irregularly shaped hypertrophies of the axis of the large diffuse inflorescences or of the transitional region between cauline and floral axes (text fig. 1). The lateral entrances or exits are not preformed apertures, as in the Physocladae, but evidently are excavated by insects. Furthermore, the domatia are not jacketed internally by invaginated layers of epidermal, cortical, and fibrovascular tissues. As shown in fig. 3, a transverse section of a myrmecodomatium of *Cordia Gerascanthus* Jacq. (Herbarium J. D. Smith no. 4365), the inflated central cylinder surrounds a large heterogeneous medulla, the large celled, succulent core of which has dried up and has been trimmed away by the ants. In other words, these structures are quite distinct morphologically from the myrmecodomatia of the Physocladae, and resemble those of the Ethiopian species of *Cuviera* and *Plectronia* (Bailey 3).

Taxonomy

Many of the discrepancies in the conclusions of various investigators who have concerned themselves with the phenomenon of myrmecophytism in *Cordia* are due to excessive generalization from limited induction. Thus, in general, SCHUMANN's conclusions are applicable to the Gerascanthi, but not to the Physocladae. Furthermore, there has been a tendency to base critical morphological and ecological observations upon plants which have not been identified with certainty, and of which no herbarium specimens are available for verification. This naturally has led to considerable confusion.

As previously stated, SCHIMPER believed that his myrmecophyte was *Cordia nodosa*, but SCHUMANN and MEZ questioned his determination and considered the plant which had hirsute stems and leaves to have been *C. hispidissima*. SCHUMANN suggested, however, that the latter species may be a variety of *C. nodosa*. Unfortunately the taxonomy of the Physocladae is a somewhat complicated problem. AUBLET (**1**) described and figured a *Cordia* which he referred to *C. Collococca*. LAMARCK (**13**) subsequently gave to this plant the specific name *C. nodosa*. DE CANDOLLE (**9**) distinguished two additional species of Physocladae, *C. miranda* and *C. hispidissima*, which are characterized by having compact, sessile inflorescences. FRESENIUS (**12**) retained these two species, and described three forms or varieties of *Cordia nodosa:* (1) *glabrior*, based on *C. formicarum* Hoffmsg.; (2) *hispidissima*, based on AUBLET's plant; and (3) *angustifolia*. It is evident from AUBLET's detailed descriptions and figures that the plant which LAMARCK referred to *C. nodosa*, and FRESENIUS to var. *hispidissima*, possessed densely hirsute stems and leaves, and small, but diffusely and dichotomously branching pedunculate inflorescences. The stems, leaves, inflorescences, and fruits of the myrmecophytic *Cordia* of the Kartabo region of British Guiana closely resemble those of this plant. In view of the fact, therefore, that AUBLET's and the writer's plants were collected in the same general region (French Guiana and British Guiana), it is reasonable to assume that they both belong to the same species.[1]

[1] The herbarium specimens collected by the writer, nos. 39, 40, 41, and 42, are deposited in the Gray Herbarium of Harvard University.

The myrmecodomatia of AUBLET's plant, of the writer's myrmecophyte, of the specimen of *C. nodosa* figured by FRESENIUS, and of two somewhat similar but subglabrous plants collected by J. A. SAMUELS in Dutch Guiana appear to be devoid of large, conspicuous, lateral, bladder-like swellings. On the contrary, the domatia of Brazilian and Bolivian Physocladae (Spruce no. 3281, Burchell no. 9637, Rusby Expedition nos. 778 and 1035, and SCHIMPER's plant) are characterized by having these unilateral, pouchlike enlargements. In view of the fact that certain systematists are inclined to group the various Physocladae together as varieties of a single species, upon the assumption that the shape and texture of the leaf, pilosity, and degree of compactness of the inflorescences are exceedingly variable, the morphology of the myrmecodomatia may prove to be of some diagnostic value. In the case of the Kartabo myrmecophyte, the form and structure of the myrmecodomatia are very constant. The writer collected material from a wide range of plants of different ages, sizes, and localities. All of the domatia were devoid of conspicuous, lateral, bladder-like enlargements. It will be of interest to determine whether these structures are equally constant in plants of other neotropical regions.

Ecology

Most of the earlier students of ant-plants considered that the structural peculiarities of the plants are originated directly by the ants. SPRUCE (**20**) and BECCARI (**6**) were forced to admit, however, that many of these abnormalities (ant-galls) have become inherited. That the myrmecodomatia of certain groups of ant-plants are produced by gall-insects rather than by ants, has been suggested from time to time by various investigators. CHODAT and CARISSO (**10**) have even formulated "*Une nouvelle théorie de la myrmécophilie*" upon the basis of the fact that they found eggs and larvae of hymenopterous parasites in immature myrmecodomatia of *Cordia glabrata*, *C. longituba*, *C. alliodora*, *C. Gerascanthus*, *C. Gerascanthoides*, and *Acacia Cavenia*. The title of their short paper might lead one to suppose that the formicaries of all myrmecophytes originate as galls. As a matter of fact, they qualify their generalization as follows: "Il y a tout lieu de penser que les formi-

cairès des autres plantes myrmécophiles, quand ils se présentent comme des renflements, ont une origine analogue."

It is to be emphasized in this connection that the mere occurrence of ants, or even of gall-insects, in hypertrophied portions of the stem or leaf does not indicate necessarily that these structures are of traumatic origin. They may be inherited peculiarities which serve as convenient nesting chambers for ants, or, during the earlier stages of their ontogeny, as suitable places for the oviposition of other insects. The large stipular thorns of many of the Mexican, Central American, and West Indian bull's-horn Acacias are not abnormalities produced by insect parasites, as evidenced by the fact that they are formed on plants which are not subject to the visitation of ants or of gall-insects. The ants are able to nest in the thorns regardless of whether they have previously been occupied by gall-insects or not. It is doubtful whether they are able to do so in the case of certain of the East African species of *Acacia*. The stipular thorns of these plants are straight and rather slender, but may be deformed at times by huge basal hypertrophies. SCHWEINFURTH (**18**), SJÖSTEDT (**19**), SCHENCK (**15**), and others are of the opinion that these apparent abnormalities actually are true galls. Although the observations of these investigators are not entirely conclusive and need to be verified by further and more detailed field studies, or by controlled experiments with plants grown from seed, it seems probable that ants are unable to utilize these thorns as formicaries unless they have previously been enlarged by gall-insects.

The myrmecodomatia of the Kartabo *Cordia*, during the earlier stages of their ontogeny, do not contain insects or parasitic fungi, and there is no histological or ecological evidence to indicate that they are originated by ants or by gall-insects. As in the case of the Ethiopian myrmecophytes, investigated by BEQUAERT (**8**) and the writer (**3**), and in that of *Tachigalia paniculata* discussed in the second paper of this series (**5**), the myrmecodomatia are inherited structures which are taken possession of by ants after their tissues have become highly differentiated. The subnodal enlargements of the Physocladae, like the stipular thorns of Mexican and Central American bull's-horn Acacias, evidently serve as formicaries without previously being modified by gall-insects.

It would not be safe to infer from this, however, that the enlargements of the fertile or vegetative axes of the Gerascanthi are formed without the intervention of parasitic insects. The irregular shape and more or less sporadic distribution of the cauline hypertrophies in this section of *Cordia* suggest that, if the myrmecodomatia are not actually originated by gall-insects, they may be considerably accentuated by them. Extensive and critical field observations are needed to determine whether the immature hypertrophies always contain eggs or larvae of parasitic insects, and if so whether homologous structures are formed on plants grown from seed, which are protected from the activities of ants and of gall-forming parasites.

Belt (7), Delpino (11), and Schimper (16) formulated the hypothesis that the structural peculiarities of ant-plants originated through the action of natural selection, as adaptations for attracting ants which protect their hosts against the attacks of phytophagous insects, particularly of the destructive leaf-cutting ants. That the guest-ants of *Cordia nodosa* in the Kartabo region of British Guiana do not afford an efficient protection against *Atta cephalotes* is illustrated in text fig. 5. Although the myrmecodomatia of this plant contained flourishing colonies of *Allomerus 8-articulatus*, many of its leaves were cut into pieces and transported to the subterranean fungus gardens of the Attine ants.

My colleague, Professor W. M. Wheeler, has identified the following ants, taken from myrmecodomatia of the Kartabo *Cordia: Allomerus 8-articulatus* Mayr, *Azteca ulei* Forel var. *cordiae* Forel, *A. instabilis* F. Smith, *A. trigona* Emery subsp. *mediops* Forel, *Neoponera unidentata* Mayr, and *Crematogaster limata* F. Smith subsp. *ludio* Forel.

The first two species are "obligatory" guests of *Cordia nodosa* or closely allied Physocladae, that is, they are known only from these plants. The remaining ants are "facultative" or more or less ubiquitous species which nest in various habitats. *Allomerus 8-articulatus* is one of the commonest guest-ants of the Kartabo *Cordia*. It is characterized by building earthern galleries which extend from the entrances of the domatia down the outer surfaces of the branches and stem, to the level of the ground.

Although all of these ants have the general formicine habit of gnawing upon the walls of their domatia, they do not excavate the

deeply sunken, pitlike or groovelike depressions which are such characteristic features of the myrmecodomatia of *Cecropia*, of *Tachigalia*, and of many of the Ethiopian ant-plants. Their failure to do so is not due to an absence of coccids, since the latter

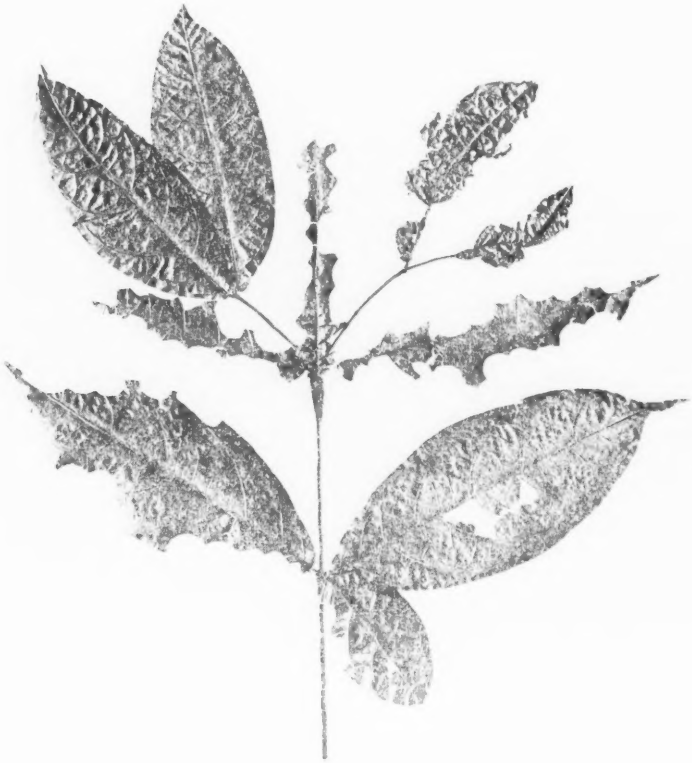


FIG. 5.—*Cordia nodosa* inhabited by *Allomerus 8-articulatus*, but partly defoliated by Attine ants.

insects usually are present in domatia which contain well established and flourishing colonies of ants. It may be significant, in this connection, that the mature myrmecodomatia of *Cecropia*, *Cuviera*, *Plectronia*, *Nauclea*, etc., are jacketed internally by compact layers of very thick walled cells, and that the coccids feed only in

gaps made in these dense tissues by the ants (BAILEY **3, 4**). In the myrmecodomatia of *Cordia*, as in the saccate appendages of the leaves of *Tococa*, the softer and more nutritious tissues are not completely isolated from the nesting chambers, and the coccids may readily insert their setae in portions of the walls which have not been cut into by the ants.

Present status of problem of myrmecophytism

The writer's (**2, 3, 4, 5**) investigations of Ethiopian and neotropical ant-plants, and a critical study of data accumulated by previous students of the problem of myrmecophytism, lead to the following conclusions.

1. The neo-Lamarckian hypotheses of SPRUCE and BECCARI, and the neo-Darwinian theory of myrmecophily formulated by BELT and DELPINO and elaborated by SCHIMPER, rest upon a series of plausible deductions or teleological inferences rather than upon extensive and critical field observations and carefully planned experimental controls.

2. There are a considerable number of tropical plants, belonging to a number of distinct families and orders, which have fistulose stems, hollow petioles, saccate leaves, enlarged stipular organs, or hollow nodal or internodal hypertrophies. In most cases these putative abnormalities are inherited structures which are formed without the intervention of parasitic organisms. Certain of these plants are provided, in addition, with extra-floral nectaries or food-bodies, that is, Perldrüsen.

3. The origin and true function of these structures are at present obscure, and deserve to be studied more intensively by plant physiologists and morphologists.

4. Insects are so abundant in tropical environments, and the competition for food and for suitable nesting chambers is so keen, that most of these structural peculiarities of plants are discovered by ants. Thus, the cauline and foliar cavities, regardless of whether they have preformed entrances or not, are found to contain more or less flourishing colonies of these ubiquitous insects. In certain cases the ants are representatives of "facultative" species which nest in various habitats, whereas in other cases they are "obligatory" guests of particular species of plants. Some of them are

more inquilines, whereas others obtain a portion of their food from the host-plants, and therefore are distinctly parasitic.

5. The myrmecodomatia of most ant-plants contain more or less luxuriant growths of fungi and numerous nematodes and coccids. The relations of these organisms to the ants and to their host plants present many interesting ecological problems.

6. The relation between the ants and the coccids and their host plants is one of the most interesting phases of myrmecophytism, and one which deserves to be studied most intensively by future investigators. Certain of the plant-inhabiting ants carefully tend the coccids and solicit and feed upon their exudates. In myrmecodomatia which at maturity are jacketed internally by compact layers of thick-walled cells, the ants excavate gaps in these dense tissues, frequently inducing the formation of a nutritive callus which is fed upon by the coccids, but not, so far as the writer has been able to determine, by the ants. Only in the case of the highly specialized *Viticicola tessmanni*, which does not tend coccids, is there evidence to indicate that the ants themselves feed upon these traumatic tissues. Are all of the coccids in myrmecodomatia introduced by the ants and carefully tended by them? This seems unlikely, for certain of the ants are not known to solicit the exudates of aphids and coccids. That the Pseudomyrminae may carve up the coccids and feed them to their larvae, even if they do not utilize them as minature milch-cows, however, has been demonstrated by the writer. It seems probable that there are various degrees of specialization in the relations between the ants and the coccids, as there are in the relations between the ants and their host plants.

7. The fungi in myrmecodomatia appear to be weeds, the aerial hyphae of which are cropped or cut back by the ants; not as food, but to prevent them from obstructing the domatia and from interfering with the brood. The spores are borne into the domatia either upon the bodies of the ants or in their infrabuccal pouches, the contents of which are voided in various portions of the formicaries.

8. The nematodes live in the detritus of the ant colonies, that is, accumulations of voided pellets, liquid feces, fragments of malaxated insects, triturated plant tissues, etc. COBB finds that certain of the

nemas are highly specialized morphologically and are adapted to feeding upon the delicate hyphae of fungi which flourish in the refuse of the ants. It remains to be demonstrated whether any of the nematodes are parasitic during their larval stages in the pharyngeal glands of the imaginal ants. It may be significant in this connection, however, that nematodes are present in the infrabuccal pellets of certain Pseudomyrminae.

9. The evidence at hand supports the conclusion that the most classical ant-plant biocoenose (Azteca-Cecropia), to which SCHIMPER devoted so much attention, is an extremely interesting case of parasitism which illustrates the remarkable adaptiveness of ants in availing themselves of the potentialities of given environments. This is shown, not only in the utilization of such structural peculiarities of the plant as the Müllerian food-bodies, prostomata, and potential domatia, but more strikingly in the structural modifications produced in the tissues of the host plant which facilitate the feeding of coccids. Thus the ants are able to obtain food from their hosts in two ways, directly from the food-bodies and vicariously through the intervention of coccids.

10. The most interesting case of parasitism is that of the highly specialized *Viticicola tessmanni* upon *Vitex Staudtii*. These obligatory guest-ants induce the formation of layers of nutritive callus, rich in protein and fats, which apparently serve as their principal source of food. In so doing they avail themselves of the structural peculiarities of their hosts in a truly remarkable manner, and exercise a very delicately adjusted control over the ontogenetic development of certain plant tissues.

11. In studying the habits of phytophagous and plant-inhabiting insects, the anatomy of the host plants deserves more careful attention than it has usually received. In the case of myrmecophytic plants, the nesting and feeding habits of the ants, and their relations to coccids and fungi in many cases are largely determined by the structure and arrangement of the various vegetative tissues during different stages in the growth and differentiation of the stem or of its appendages.

This investigation was conducted under a grant from the American Association for the Advancement of Science. The writer

wishes to thank WILLIAM BEEBE for numerous courtesies during his visit to the Tropical Station of the New York Zoological Society. DR. ORLAND E. WHITE very kindly sent to the writer specimens of various myrmecophytes collected by the Rusby Expedition.

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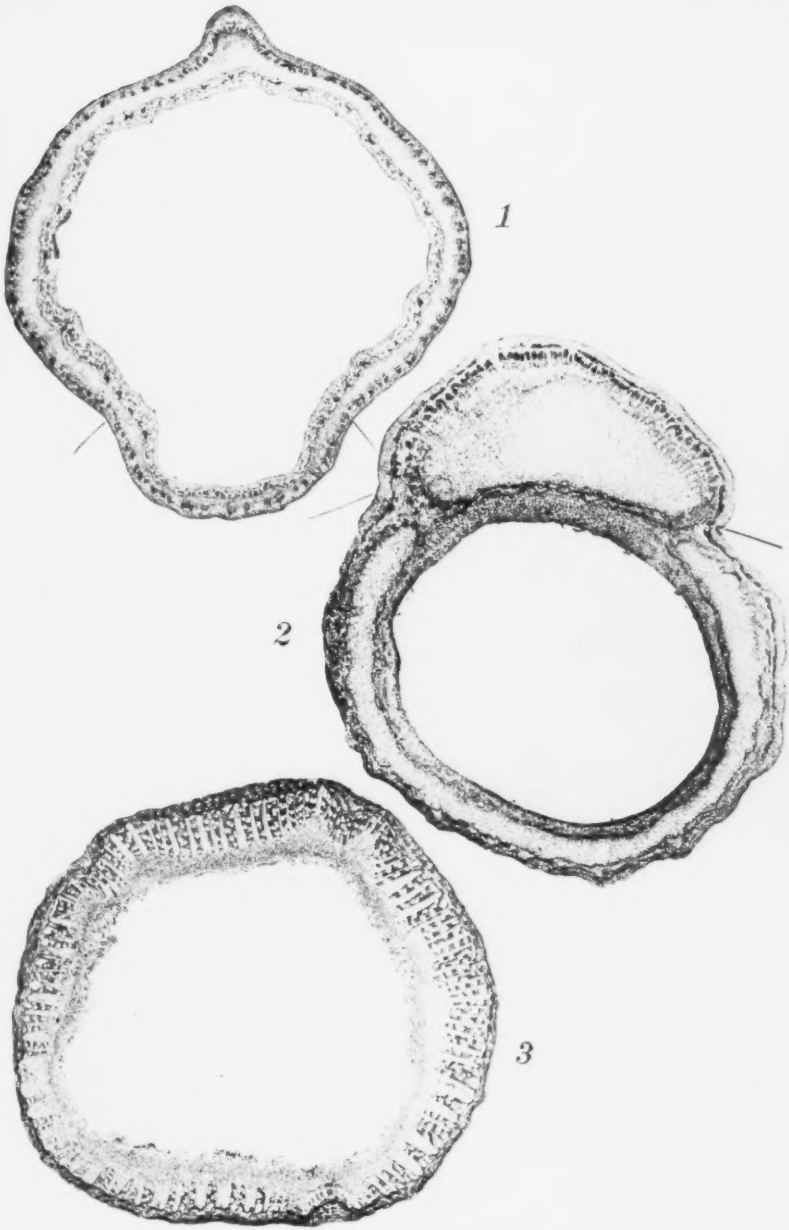
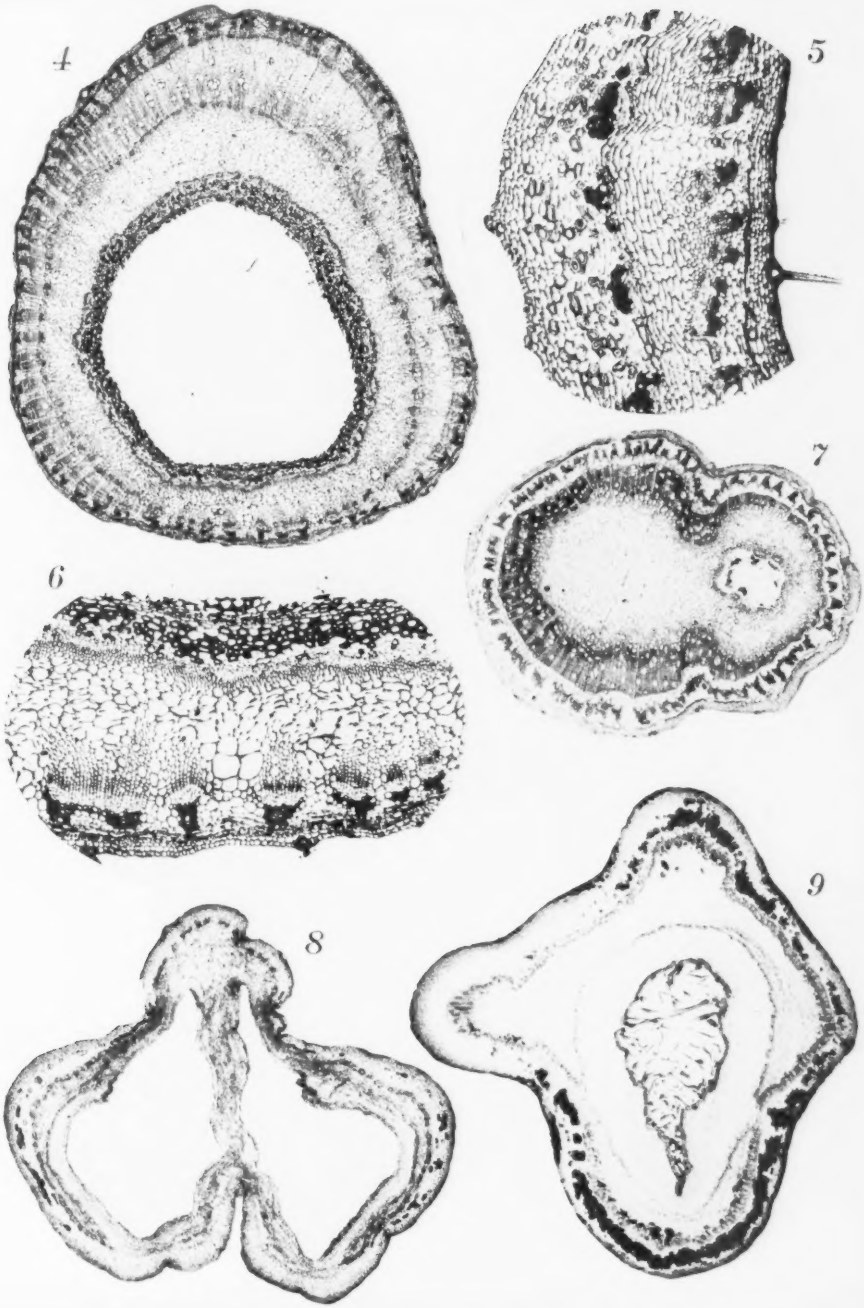


BAILEY on ANT-PLANTS



BAILEY on ANT-PLANTS

EXPLANATION OF PLATES VI, VII

FIG. 1.—*Cordia nodosa:* transverse section of myrmecodomatium, showing centripetal and centrifugal fibrovascular cylinders; ×7.

FIG. 2.—*Cordia* species: transverse section of myrmecodomatium; inner fibrovascular cylinder is divided into two arcs which have united with two corresponding arcs of outer fibrovascular cylinder; ×14.

FIG. 3.—*Cordia Gerascanthus* Jacq. (Herbarium J. D. Smith no. 4365): transverse section of myrmecodomatium, showing medullary cavity and single fibrovascular cylinder; ×11.

FIG. 4.—*Cordia nodosa:* transverse section of base of old woody myrmecodomatium, showing inner, rudimentary fibrovascular cylinder and thick layer of xylem in outer fibrovascular cylinder; ×13.

FIG. 5.—*Cordia nodosa:* portion of fig. 1 more highly magnified; ×50.

FIG. 6.—*Cordia nodosa:* lower portion of fig. 4 more highly magnified; ×42.

FIG. 7.—*Cordia hispidissima:* (Burchell no. 9637): transverse section of base of myrmecodomatium, showing lower extremity of invagination; ×14.

FIG. 8.—*Cordia nodosa:* transverse section of immature, compound domatium, formed by invaginations from axils of two of leaves of false verticil; ×11.

FIG. 9.—*Cordia nodosa:* transverse section of apical portion of immature myrmecodomatium, showing four leaf traces and central cavity jacketed by cuticularized epidermis and numerous trichomes; ×16.

LEAVES OF TRIGLOCHIN[1]

AGNES ARBER

(WITH PLATES VIII–X)

Introduction

In a previous paper (4) a general survey was made of the leaf structure of the group of families comprised in the cohort Helobieae. While examining the leaves of one of these families, Scheuchzeriaceae (Juncaginaceae), I became interested in *Triglochin*, because this genus includes both leaves of more or less radial structure, and also typical "ribbon" leaves. The present paper describes the results of a further study of the leaves of *Triglochin*, which has been rendered possible by the generosity of J. H. MAIDEN, of the Botanic Gardens, Sydney, New South Wales, and of L. HAUMAN, of the Museo Nacional de Historia Natural, Buenos Aires, who have been most kind in supplying me with Australian and South American species. I am also indebted for material to the Director of the Royal Botanic Gardens, Kew, and to A. C. SEWARD.

Leaf structure

According to BUCHENAU (10), *Triglochin* includes two subgenera, *Eutriglochin* with united carpels, and *Cycnogeton* in which the carpels are free. *Eutriglochin*, to which the two British species *T. maritima* and *T. palustris* belong, was treated by BUCHENAU as containing twelve species, but the number has been increased somewhat in the twenty years that have since elapsed. On the other hand, *Cycnogeton* includes only one species, a native of Australasia, *Triglochin procera*. I have been able to examine this plant, and also nine species belonging to the subgenus *Eutriglochin*. In the following brief account of the leaf structure of the genus, the species, so far as possible, are taken in the order in which they are placed by BUCHENAU.

[1]This paper represents part of the work carried out during the tenure of a Keddey Fletcher-Warr Studentship of the University of London.

Subgenus Eutriglochin

Triglochin maritima L.—The leaf of this species has a sheathing base and a succulent linear limb. The sheath is continued upward into a ligule (fig. 1, *lig*). Fig. 2*A* shows the leaf sheath region in transverse section. Besides the normal bundles with their fibrous sheaths, there are occasional small strands (*f*) consisting of fibers alone. Fig. 2*B* represents a section of the limb of another leaf on a slightly smaller scale. The median bundle is placed in the usual way, with xylem above and phloem below, but the smaller peripheral bundles all have their xylem directed inward, so that in those on the adaxial side of the leaf the xylem lies below the phloem. I have indicated elsewhere (**1**) the reasons that lead to the interpretation of leaves showing this general type of structure as petiolar phyllodes. The relation of the bundles in sheath and limb has been described in detail by Hill (**12**). The changes in anatomy toward the apex of the limb of *T. maritima* can be followed in fig. 3*A*–*C*. Fig. 3*A* shows the condition reached as the tip is approached. All the lacunar tissue, which in fig. 2*B* occupies the center of the section, has disappeared, and the whole mesophyll is formed of palisade parenchyma. The vascular system is reduced until it consists of little more than the three main strands, lying in one horizontal plane. The bundle sheaths are lost, and there is a great increase of xylem relative to phloem. Fig. 3*B* shows the structure of the median bundle at a level a little lower than that of fig. 3*A*. There is a mass of primary xylem (xy_1), consisting of relatively large elements, and also a conspicuous development of secondary xylem (xy_2), composed of elements of smaller caliber, arranged very definitely in radial rows, and markedly isolated from the primary xylem. Higher up, however, there is less separation between the primary and secondary wood. The increase in the primary xylem toward the leaf apex probably is to be attributed to sliding growth. As the tip is approached, the bundles fuse into one mass (fig. 3*C*), the connection between the bundles being established by branches uniting the secondary xylem of the three bundles. Neither in the serial sections from which fig. 3*A*–*C* was drawn, nor in a longitudinal series through the apex of another old leaf, was any indication found of an "apical opening" or apical

water pores. *T. maritima* thus agrees with *T. palustris*, in which BUCHENAU (**10**) reports the absence of an apical opening.

Fig. 4*A*, *B* shows sections of the sheath and limb of a leaf of *T. maritima* from Patagonia, for comparison with fig. 2*A*, *B*, drawn from British material. In fig. 4*A*, *B* the lacunae of the mesophyll are not outlined. The small size of the leaf (here represented on a larger scale) and the greater development both of the palisade parenchyma and fibers are very noticeable features in the Patagonian material. Instead of the occasional minute fibrous strands, which occur in the leaf sheath of the British plant, the Patagonian specimen shows six strands consisting exclusively of fibers, taking the place of the vascular bundles in the marginal region of the sheath. Without a further study of the Patagonian form, however, it is impossible to say whether these differences are constant.

In fig. 5*A*–*D* four sections are drawn, selected from a series passing upward from below through an axillary bud (*lb*) of *T. maritima*, inclosed in its prophyll (*pr*). According to BUCHENAU'S (**8**) observation, which REUTER (**14**) has recently confirmed, the lateral vegetative shoots in the axils of the upper leaves begin with a completely developed foliage leaf, while it is only the vegetative shoots in the axils of the lower leaves that have two-keeled prophylls. The bud here described belonged to the latter type. In the prophyll the main lateral bundles (*ml*) are the most conspicuous strands. Fig. 5*A* shows the origin of four of the curious "intravaginal squamules" (*a*, *b*, *c*, *d*), whose occurrence on the surface of the internode, just above the level of exsertion of each leaf, has already been recorded for this species and for *T. palustris* (**13**). I have discussed these squamules in a recent paper (**7**), and therefore now only allude to the fact that the squamule *d* is unusual in arising from the actual line of junction of prophyll and bud axis, whereas the normal state of things in *Triglochin* (the origin of the scales from the internodal surface) is exemplified in fig. 5*A* (*a*, *b*, *c*), and in α and β, belonging to the next set of squamules (fig. 5*B*). Fig. 5*B*, *C* shows that the prophyll has a closed sheathing region at the base. In fig. 5*D* the sheath is open on the side remote from the parent axis. As in the foliage leaf, there is a tendency in the

prophyll for the replacement of vascular bundles by strands of a purely fibrous character. Fig. 5*E*, *F*, *G* represents three bundles from fig. 5*D* on a larger scale, to show the grades of reduction from a bundle (fig. 5*E*), which includes xylem, phloem, and a fibrous sheath, through a bundle (fig. 5*F*) in which the xylem is reduced to one element only and with little phloem, to such a strand as that shown in fig. 5*G*, which consists exclusively of a small group of fibers.

TRIGLOCHIN MARITIMA L. var. DESERTICOLA Buch.—One well marked variety of *T. maritima* is recognized by BUCHENAU. Through the kindness of Dr. HAUMAN I have been able to examine the leaf structure of this variety from Las Cortaderas, Cordillères de la Rioja. It has, he tells me, the habit of a compact "cushion plant." Fig. 6*A*, *B* shows the anatomy of the leaf sheath and limb in this variety. No definite palisade parenchyma was visible, but this may have been due to the difficulty of getting complete recovery of the tissues in dried material. The most interesting feature of the anatomy is the coexistence, in the same leaf limb, of bundles showing two types of structure, collateral and amphivasal. The more important bundles are of the normal type, but in some of the smaller strands the xylem surrounds the phloem. Fig. 6*C* shows the two bundles (marked *x* in fig. 6*B*) on a larger scale. The lefthand bundle is collateral, with a fibrous sheath (*f*) on the phloem side, while in the righthand bundle there is a discontinuous wreath of tracheids inclosing the phloem.

TRIGLOCHIN CONCINNA Davy.—This species is closely related to *T. maritima*. Its leaf structure is shown in fig. 7. The small peripheral bundles all have xylem directed inward, and succeeded externally by phloem and a patch of fibers. The xylem forms a crescent, or even an almost complete ring, including the phloem.

TRIGLOCHIN PALUSTRIS L.—This species closely resembles *T. maritima* in the main lines of its leaf structure. Fig. 8 shows the transverse section of the limb. At first glance the small peripheral bundles look almost as if they were amphivasal, but in reality there is a crescent of xylem on the inner side, and the ring is completed by fibers on the outer side. Fig. 9 represents a transverse section of the limb of a leaf of the same species from the Andes.

The leaf is smaller and the bundles are fewer than in the English material. Moreover, in the leaf from the Andes the mesophyll is all lacunar, without differentiated palisade parenchyma, and the fibers surrounding the bundles have thicker walls. A characteristic feature of *T. palustris* is the occurrence of stolons (fig. 10*A*), each of which bears scale leaves and terminates in a small bulb (**8**). This is formed of a thickened leaf (fig. 10*D*) inclosing a series of smaller leaves, also thickened and containing starch. The entire mesophyll of the leaf represented in fig. 10*D*, and even the epidermis on both sides, consists of cells densely packed with starch grains. The storage leaves are inclosed in thin scale leaves (fig. 10*B*), the veins of which are very fibrous. One of the strands toward the margin of the thin scale leaf is shown enlarged in fig. 10*C*; it is composed exclusively of fibers. The upper epidermis is strongly thickened, and the walls communicating with neighboring elements are pitted. Both the storage and outer scale leaves of the bulb correspond, morphologically, with the sheathing region of the ordinary foliage leaf, without its limb.

TRIGLOCHIN STRIATA Ruiz et Pav.—Fig. 11 shows a transverse section of the leaf limb of a specimen of *T. striata* from Natal. The small bundles toward the adaxial side have the xylem directed downward. In fig. 12 a corresponding leaf limb of *T. striata* var. *α triandra* from New Zealand is represented. In this case there are no inverted bundles toward the adaxial side of the leaf. Fig. 13*A*–*C* shows the leaf structure of another variety, *T. striata* var. *β montevidensis*. The sheath (fig. 13*A*) is succeeded by a limb (fig. 13*B*) in which, in the case of the leaf drawn, two of the minor bundles (*x*) are amphivasal. Fig. 13*C* shows one of these amphivasal bundles on a larger scale. An example of this variety, figured by SEUBERT (**16**), has ribbon leaves, and in the specimen that I examined, the limb, so far as could be told from the herbarium material available, was also ribbon-like, as is shown by the somewhat flattened form of the transverse section (fig. 13*B*).

TRIGLOCHIN BULBOSA L.—This species is widely distributed in the Mediterranean region, and also occurs in South Africa. Most of the leaves have the usual sheathing base, succeeded by a narrow limb, but some are reduced to leaf sheaths alone. Herbarium

specimens have been seen with leaves at least 7 cm. long. Fig. 14*A*–*D* shows a series of transverse sections of a normal foliage leaf, from Crete, passing from the extreme top of the sheath (*A*) to near the apex of the limb (*D*). At the level of *D*, which is close to the tip of the leaf, the main laterals are much richer in xylem than the median bundle. The latter, however, is distinguished from the strands on either hand by the presence of a sclerized bundle sheath. Fig. 15*A* shows a transverse section of another leaf, on a larger scale than fig. 14. The three main bundles are of the normal collateral type, but in the case of the smaller bundles the xylem more or less completely surrounds the phloem. Fig. 15*B* shows the group of bundles inclosed in the dotted line in fig. 15*A*, more highly magnified. The largest bundle (one of the main laterals, *ml*) has an ordinary *V*-shaped xylem group, and is inclosed in a fibrous sheath (*f*), while each of the four small bundles (*x*) have a ring of xylem surrounding the phloem. Fig. 16*A* shows the transverse section of the limb of another leaf, in which there is a median bundle (*mb*), two main laterals (*ml*), and four minor amphivasal bundles (*x*). One of these amphivasal strands is shown on a larger scale in fig. 16*B*. To try to discover the mode of origin of the amphivasal structure, serial sections were cut through the leaf sheath of *T. bulbosa*. Fig. 17*A*, *B*, *C* shows a bundle, which, when followed in this way, at first proved to be collateral (fig. 17*A*). As the upper limit of the sheath was approached, however, the xylem elements crept round the phloem, until finally they completely inclosed it (fig. 17*C*). Another point, studied by means of serial sections of the leaf of *T. bulbosa*, is the behavior of the bundles in the apical region of the limb. Fig. 18*A* shows that, as the apex is approached, there is a tendency for the bundles to take up a position in one horizontal plane, while the individual strands become more or less amphivasal. The bundles next merge into an irregular dorsiventral complex, with the phloem chiefly on the lower side. By the level reached in fig. 18*B* (which is on a larger scale than fig. 18*A*), the phloem appears to have died out. Fig. 18*C*–*E* shows the gradual disappearance of the tracheids, the extreme apex of the leaf consisting of mesophyll and epidermis alone. A longitudinal section of a similar leaf tip is seen in fig. 19.

TRIGLOCHIN LAXIFLORA Guss.—This species is closely related to *T. bulbosa;* in fact, it is regarded by BUCHENAU (9) as merely a "petite espèce," which has become separated from *T. bulbosa* by difference in flowering time. Its leaf structure (fig. 20*A*, *B*) so far as can be told from examining a fragment of one leaf, shows no essential difference from *T. bulbosa*, but the palisade tissue is more conspicuous than in the latter species, and no amphivasal bundles have been observed. The leaves, like those of *T. bulbosa*, are sometimes reduced to leaf sheaths without limbs.

TRIGLOCHIN STOWARDII N.E.Br., T. ANDREWSII N.E.Br., and T. MINUTISSIMA F. v. Muell.—These three small species from Australia agree in possessing a very slender leaf limb and a delicate skeletal system. In each case I have only been able to examine one leaf. In *T. Stowardii* (fig. 21) the median bundle has a sheath in which the elements are strongly thickened on the inner side, but no such thickened sheath occurs in the case of the other bundles. In *T. minutissima* (fig. 23) there are a few fibers, not very thick walled, associated with the bundle. *T. Andrewsii* (fig. 22) has the most delicately organized leaf met with in *Eutriglochin;* the only sclerized tissue is the epidermis, whose outer wall is thickened.

SUBGENUS CYCNOGETON

Triglochin procera is an aquatic plant, occurring in Australia and Tasmania. The leaves are of the ribbon type, and may attain considerable length. Among some material kindly secured by J. H. MAIDEN from Glenfield, George River, near Sydney, New South Wales, there was more than one leaf over a meter long. Fig. 24 shows the junction of sheath and limb in one of these ribbon leaves. The ligule, which occurs in *Eutriglochin* (for example, *T. maritima*, fig. 1), is absent. The sheath wings (*sh*) are membranous, and such vascular tissue as they contain is comparatively inconspicuous. In their structure the leaves of *T. procera* show a great range of variation. Fig. 25*A*–*E* represents a series of transverse sections from below upward through a leaf of a plant from Glenfield, in which the ribbon leaves were large and strongly formed. Fig. 25*A* represents the basal sheathing region. The narrow band of mesophyll between the upper epidermis and the lacunae consists of

cells not elongated at right angles to the surface of the leaf. On the other hand, in fig. 25*B*, which represents the main part of the leaf, the palisade is several layers deep, but the individual cells are not greatly elongated. There are about half a dozen main bundles, and also a large series of smaller bundles, placed nearer the adaxial surface of the leaf, just below the palisade. All the bundles are normally orientated. Fig. 25*C–E* shows sections near the apex. The zone (*m*) in *C* is a band of mesophyll tissue which has lost most of its palisadic character. At this stage there is an increase in the amount of xylem, and most of the bundles are cut obliquely in transverse sections, since they are beginning to converge. Fig. 25*D* shows considerable bundle fusion, and a further development of wood. In fig. 25*E* the bundles have united into two elongated vascular bands, consisting chiefly of tracheal elements. As in *T. maritima* and *T. bulbosa*, no sign of any apical opening was found. The stomates of the leaf epidermis are seen in fig. 26. Fig. 29*A–D* shows a somewhat different type of leaf belonging to the same species; the specimen in question came from Manly, New South Wales. The limb (*C*) is less conspicuously of the ribbon type, and the section represented included four irregular series of bundles. One bundle on the adaxial side (*ib*) was oriented with the xylem downward. Fig. 28*A*, *B* shows yet a third variation in leaf structure within the same species. These represent the sheath and limb of an extremely small and delicate ribbon leaf from Crawford River, New South Wales. The limb (*B*) has no differentiated palisade tissue, and is traversed by one series of bundles only. Details of the leaf bundles of *T. procera* are shown in figs. 27, 29*B*, *D*. As in *Eutriglochin*, the bundle sheath, in cases where it is one layered, consists of elements with a thickened inner wall (figs. 27 and 29*D*).

Comparison and Summary

Amphivasal bundles.—As regards vascular anatomy, the most noticeable point is the occurrence of amphivasal structure ("disposition périphérique" of Chauveaud 11) in some of the minor leaf bundles of certain species of *Eutriglochin*. These amphivasal bundles, in which the tracheal elements inclose the phloem, have

been observed in *Triglochin bulbosa* (figs. 15*A*, *B*, and 16*A*, *B*, 17*A*–*C* and 18*A*); *T. maritima* var. *deserticola* (fig. 6*B*, *C*); and *T. striata* var. *β montevidensis* (fig. 13*B*, *C*). Similar amphivasal strands have been observed in the upper part of the coleoptile in *Avena sativa* and certain other grasses, and in the scutellum of *Hordeum vulgare*. In these cases the bundles are collateral at the base, becoming concentric higher up by the creeping of the xylem elements round the phloem. The amphivasal bundles of the *Triglochin* leaf seem to have a similar mode of origin (fig. 17*A*–*C*). Concentric bundles are a familiar feature of monocotyledonous rhizomes, and they have been described by HILL (**12**) for the axis of *Triglochin*. They are also known from the scutellum of *Zea Mays* (**15**), but, so far as known, there has not hitherto been any record of their occurrence in the foliage leaves of monocotyledons.

FIBROUS STRANDS.—Another point in the vascular anatomy of *Triglochin* is the occasional replacement of normal bundles by strands consisting exclusively of fibers. Such strands are to be found in the scale leaves inclosing the bulbs of *T. palustris* (fig. 10*B*, *C*), and may also be observed in the base of the foliage leaf of the British *T. maritima*. In material of *T. maritima* from the Andes, however (fig. 4*A*), they are developed on a more conspicuous scale, and it is clear from their position that they are degraded representatives of the bundles which, in the case of the British plant, have a normal equipment of xylem and phloem. Fig. 5*E*, *F*, *G* shows gradations between a normal vascular bundle, in which the fibers merely form a sheath on the phloem side, to a strand consisting exclusively of fibers. The bundles in question occurred in a prophyll of *T. maritima*, but such gradations are not peculiar to this genus, and can be paralleled in other monocotyledonous leaves (**1**).

STRUCTURE OF LEAF APEX.—I have made some study of the anatomical changes met with in certain species of *Triglochin* as the apex of the limb is approached. These changes are illustrated for *T. maritima* in fig. 3*A*–*C*, for *T. bulbosa* in figs. 18*A*–*E* and 19, and for *T. procera* in fig. 25*C*–*E*. A marked feature in these three cases is the relative increase in the development of xylem as the apex is approached. Increase in wood and sclerized tissue at the

extreme apex of the limb, indeed, is a common phenomenon in monocotyledonous leaves (**3, 5**). In *Triglochin* at least, it seems impossible to interpret this excessive xylem development on teleological lines, since there is no indication of any "apical opening," or of terminal water pores. In the case of *T. maritima*, secondary xylem is formed in the apical region on a scale which is unusual for the foliar bundle of a monocotyledon (fig. 3*B*). The ultimate fusion of the bundles is brought about by tracheal branches which pass from one of the masses of secondary xylem to the next. In previous papers (**2, 6**) it has been shown that in the leaves of monocotyledons the tracheal elements of the cross connections, uniting the longitudinal veins, tend to be attached exclusively to the secondary xylem of the longitudinal bundles, in cases in which a distinction between primary and secondary wood can be recognized. The behavior of the xylem in the leaf tips of *T. maritima* thus furnishes another example of the part played by secondary xylem in lateral connections between the foliar bundles of monocotyledons.

Prophylls and "squamulae intravaginales."—Although previous investigators have described the prophyll of the lateral bud (**8, 14**) and the origin of the "squamulae intravaginales," which are found among the young leaves of *Triglochin* (**12, 13**), these structures have not been figured to show their anatomical relations, so I have drawn transverse sections of a lateral bud of *T. maritima* at different levels (fig. 5*A*–*D*), since these sections include both the organs in question. The squamules have been discussed more fully elsewhere (**7**).

Ribbon leaves.—The leaves of *Triglochin striata* var. *montevidensis* (*Eutriglochin*) and of *T. procera* (*Cycnogeton*) belong to the "ribbon" type. I have not been able to make a thorough examination of the former, but have had the opportunity of cutting sections of a number of specimens of *T. procera* from different localities. The leaves of this species, although conforming to the general aquatic ribbon type, present a series of structural variations, which, however, appear to grade into one another. Fig. 25 shows a very well developed ribbon leaf, with adaxial palisade parenchyma, and numerous bundles in two series. The leaf probably floated upon the water surface. Fig. 28*A*, *B* represents a more delicate ribbon

leaf, which most likely was entirely submerged. It has no palisade parenchyma, and the vascular system of the limb, at the level shown, consists of six bundles in a single series. It closely resembles a small ribbon leaf of *Sagittaria sagittifolia* (4). On the other hand, fig. 29*A*, *D* shows a solider type of leaf, whose structure recalls that of the Sagittarias of the *teres* group (4), or of the petioles of those leaves of *S. sagittifolia* which are transitional in structure between ribbon leaves and leaves with a differentiated limb (4). The parallelism between the ribbon leaves in the Scheuchzeriaceae and Alismataceae, referred to in a previous paper (4), thus finds expression in the anatomy as well as in the more obvious external characters.

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EXPLANATION OF PLATES VIII–X

Throughout, xylem (*xy*) shown in black, and in high power drawings walls of elements indicated in solid black; phloem (*ph*) in white; fibers (*f*) dotted, and in high power drawings thickness of wall indicated by double lines; *ep*, epidermis; *hyp*, hypoderm; *pal*, palisade parenchyma; *ib*, bundles with xylem directed downward; *mb*, median bundle; *ml*, main lateral bundle; *lac*, lacunae, the outlines of which are in some but not all cases indicated by dotted line. Since majority of sections were cut from dried material, there is often some distortion owing to incomplete recovery of original form, so that the transverse section is more contracted and less symmetrical than it would have been in life.

PLATE VIII

FIGS. 1–5.—*Triglochin maritima*. Fig. 1: sheath (*s*), and base of limb (*l*), with ligule, *lig* (slightly reduced). Fig. 2: *A*, transverse section of leaf sheath from Norfolk (×23), *f*, small strands consisting of fibrous elements alone; *B*, transverse section of limb of another leaf from Norfolk (×14). Fig. 3 *A–C:* transverse sections from microtome series, from below upward, through apical region of old leaf from Wells, Norfolk; *A* (×23); *B*, median bundles of section a little below *A* (×318), xy_1, primary xylem, xy_2, secondary xylem; *C*, transverse section close to apex (×23). Fig. 4 *A*, *B:* transverse section of sheath (*A*) and limb (*B*) of leaf from Patagonia (×47). Fig. 5 *A–D:* series of transverse sections from below upward through lateral bud (*lb*) with its prophyll (*pr*), and first leaf after prophyll (l_1). Parent axis would lie toward foot of page. "Squamulae intravaginales" (*a*, *b*, *c*, *d*, α, β) shaded. Fig. 5 *E–G:* three of bundles from *D* (×318); *E* is bundle marked with arrow on lower side of section; *F*, bundle marked *x;* *G*, bundle marked with arrow on upper side of section, consisting exclusively of fibers.

FIG. 6 *A–C.*—*Triglochin maritima* var. *deserticola* (co-type from Herbarium of University of Cordoba): *A*, transverse section of sheath (×47); *B*, transverse section of limb (×47); two bundles (*x*) in *C* show amphivasal character of that to the right hand (×318).

FIG. 7.—*Triglochin concinna*, California, Kew Herbarium: transverse section of limb (×47).

PLATE IX

FIGS. 8–10.—*Triglochin palustris*. Fig. 8: transverse section of limb of leaf from Surrey (×23). Fig. 9: transverse section of limb of leaf from Andes (×23). Fig. 10: *A*, base of plant (×0.5), Kew Herbarium, with two stolons (*st*) terminating in bulbs (*b*), other stolons omitted, *l*, young leaves, *fi*, fibrous remains of older leaves; *B*, transverse section of sheathing leaf inclosing bulb (×14); *C*, region including strand (*f*) in *B* (×318), *uc*, upper epidermis, *le*, lower epidermis; *D*, transverse section of thickened leaf of bulb (×14).

FIG. 11.—*Triglochin striata:* transverse section of limb of leaf from Natal, Kew Herbarium (×23).

FIG. 12.—*Triglochin striata* var. α *triandra:* transverse section of limb of leaf from New Zealand, Kew Herbarium (×23).

FIGS. 13 *A–C.—Triglochin striata* var. β *montevidensis*, from Mendoza, Argentina: *A*, transverse section of leaf sheath (×47); *B*, transverse section of limb of another leaf (×47), bundles (*x*) are amphivasal; *C*, amphivasal bundle from section similar to *B* (×318).

FIGS. 14–19.—*Triglochin bulbosa*. Fig. 14 *A–D*, material from Crete, Kew Herbarium, series of transverse sections from extreme top of sheath (*A*) to near apex (*D*) (×23). Fig. 15 *A*, *B*, transverse sections of another leaf from Kew Herbarium; *A*, transverse section of limb, including ten amphivasal bundles (×47); *B*, group of bundles inclosed in dotted line in *A* (×193); tracheal elements and fibers alone represented. Fig. 16: *A*, transverse section of limb of another leaf (×14), four bundles (*x*) are amphivasal; *B*, one of amphivasal bundles in *A* (×318). Fig. 17 *A*, *B*, *C*, a minor bundle followed upward from below in serial sections through leaf sheath (×193). Fig. 17 *C*, near upper limit of sheath, xylem has inclosed phloem, tracheal elements are indicated, but phloem is merely outlined. Fig. 18 *A–E*, transverse sections from microtome series passing upward from below through apex of leaf; *A* (×47); *B–E* (×77). Fig. 19, radial longitudinal section from microtome series passing through leaf apex (×318).

PLATE X

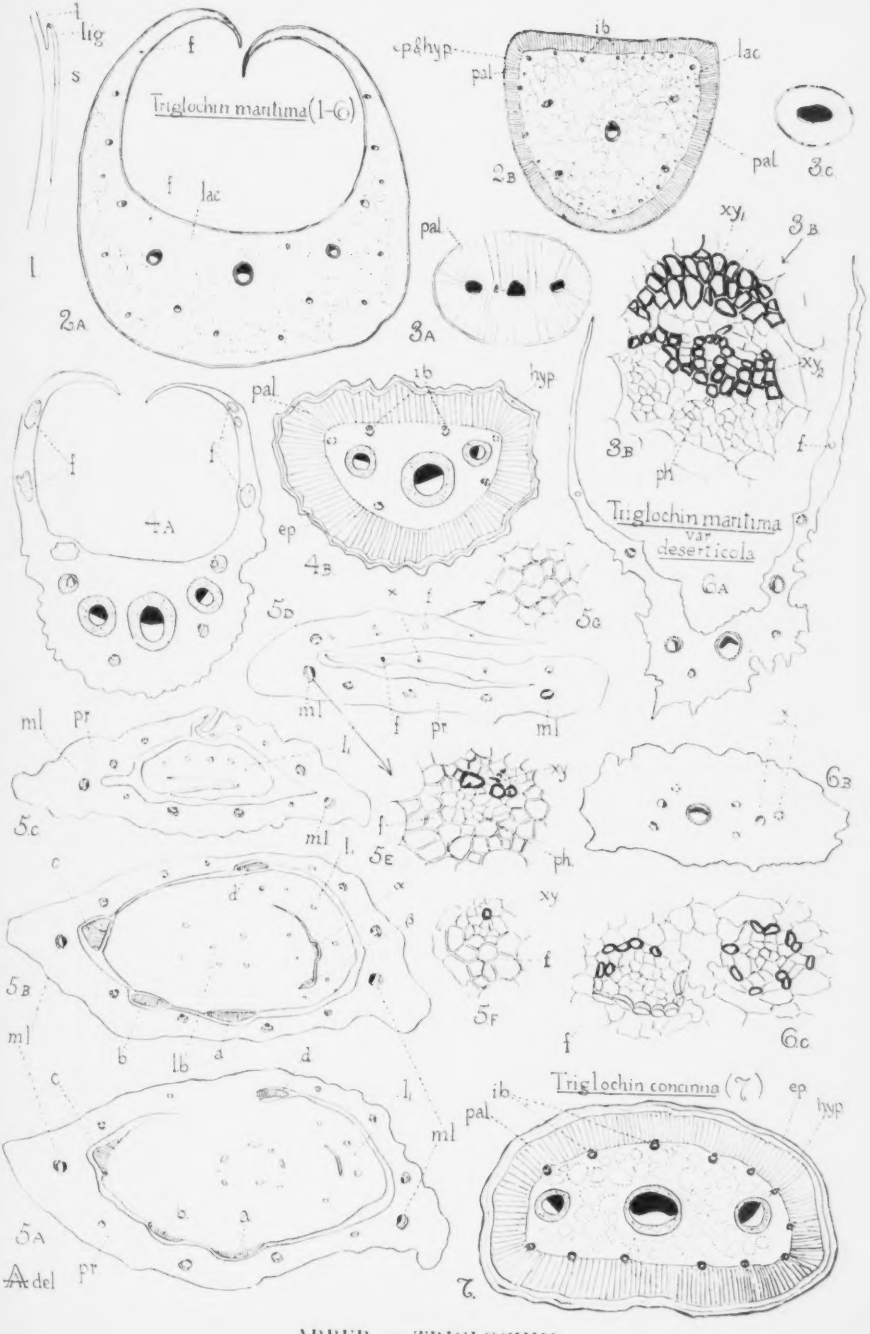
FIGS. 20 *A*, *B.—Triglochin laxiflora:* two transverse sections of one leaf from Malta, Kew Herbarium (×23); *B*, nearer apex.

FIG. 21.—*Triglochin Stowardii:* West Australia, Kew Herbarium; transverse section of limb of leaf (×47).

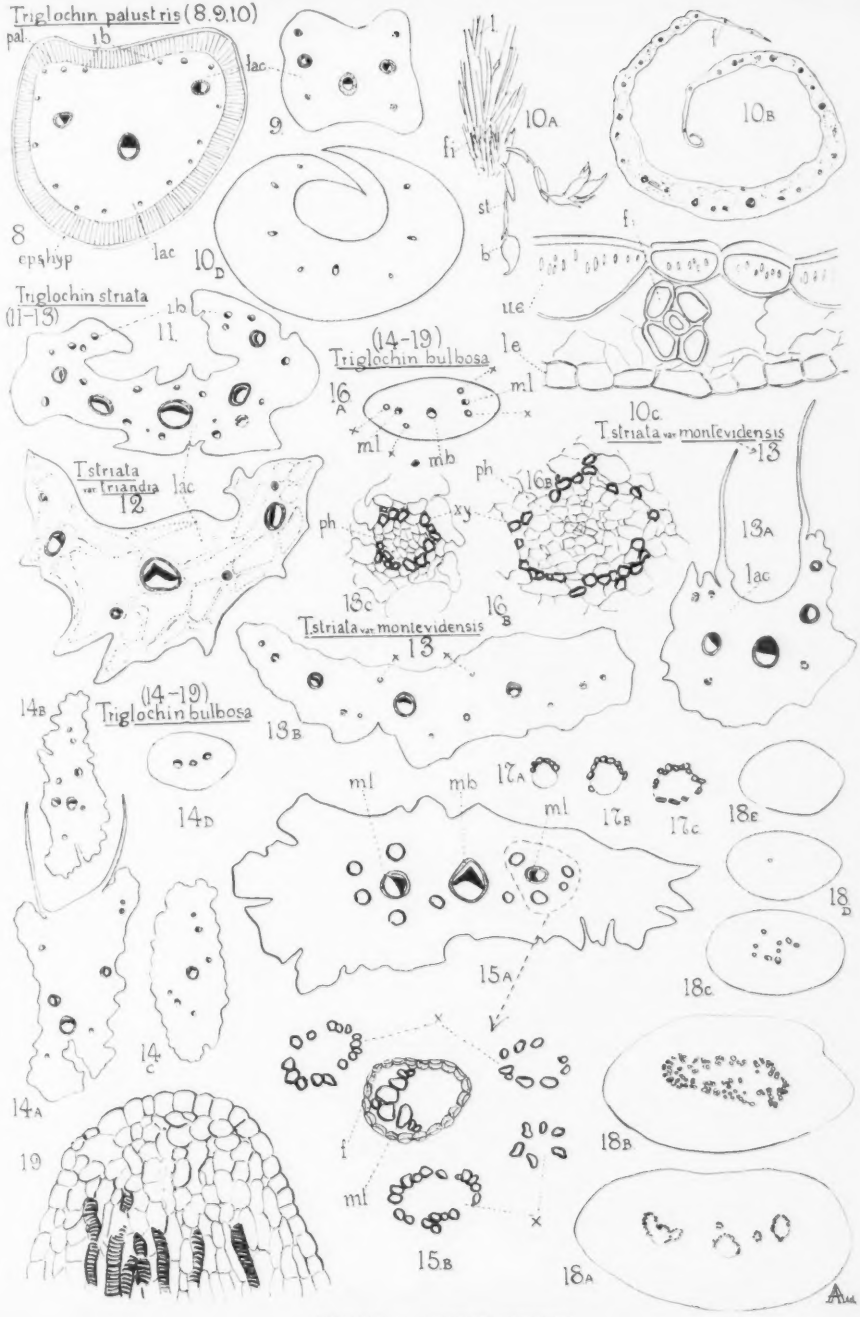
FIG. 22.—*Triglochin Andrewsii:* West Australia, Kew Herbarium; transverse section of limb of leaf (×47).

FIG. 23.—*Triglochin minutissima:* George Town, Kew Herbarium; transverse section of limb of leaf (×47).

FIGS. 24–29.—*Triglochin procera*. Fig. 24, junction of sheath and limb of leaf from Glenfield, George River, near Sydney (rather more than ×0.5); *sh*, sheath wings. Fig. 25 *A–E*, series of transverse sections from below upward, through leaf from Glenfield (×14); *A*, basal sheathing region; *B*, main part of ribbon limb, divided because too wide for page. Fig. 26, epidermis and stomates of leaf from Glenfield (×193); guard cells dotted for distinctness. Fig. 27, median bundle from transverse sections of leaf (locality unknown); *px*, protoxylem space; *bsh*, bundle sheath (×193). Fig. 28 *A*, *B*, transverse sections of leaf from Crawford River, New South Wales; *A*, sheath (×14); *B*, limb (×23). Fig. 29 *A–D*, transverse sections of leaf from Manly, New South Wales; *A*, *C*, sheath and limb (×23); *B*, bundle marked (*x*) in *A* (×193); *D*, marginal bundle, such as that marked (*x*) in *C* (×193).



ARBER on TRIGLOCHIN



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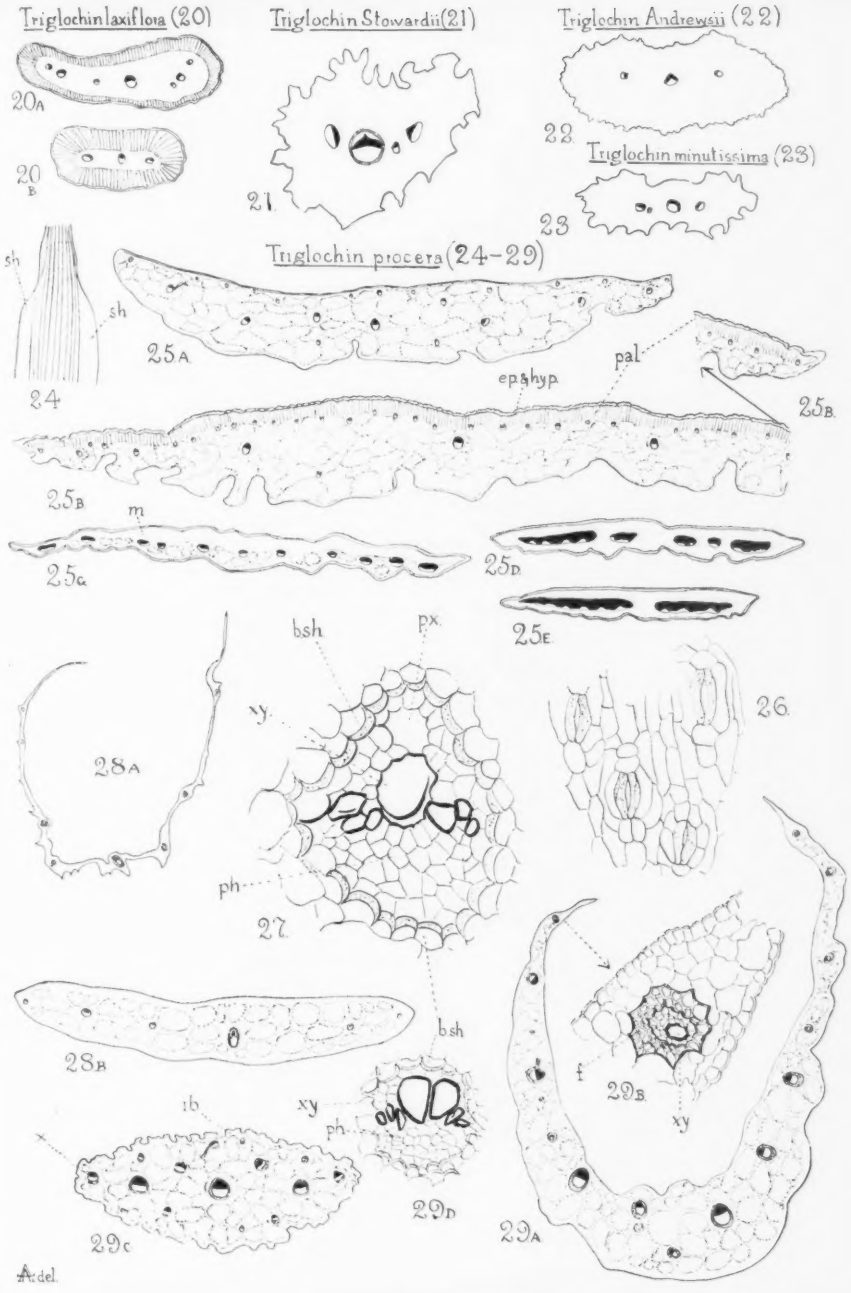

ARBER on TRIGLOCHIN

INFLUENCE OF SODIUM ARSENITE ON MICROFLORA OF SOIL

J. E. Greaves and E. G. Carter

Arsenic is present in many rocks and waters (**14**), and it has been found in varying quantities in a number of virgin prairie soils (**2, 12**), and soils of the arid regions often contain it. Some cultivated orchard soils contain it to the extent of 138 parts per million (**5**). The quantity of water-soluble arsenic in such soils varies with the total arsenic applied, and also with the quantity of alkali salts present. There is also being applied to soils in some districts arsenites (**4**) which are conceded to be much more toxic to plants than are the arsenates (**21**). The results obtained in a study of the influence of the arsenates on the microflora of the soil have been published (**6, 7, 9**), hence it is the province of this paper to consider the influence of sodium arsenite upon the soil microflora.

Method

The work was conducted on three different soils. Soil no. 1 was a fine sand of sedimentary origin and well supplied with all the essential plant elements except nitrogen, which was low. It contained a liberal supply of calcium and magnesium carbonate, but the organic content was very low. Soil no. 2 was a calcareous silt loam (**10**) which was exceptionally rich in phosphorus and potassium, but low in nitrogen and organic matter. The calcium and magnesium contents were exceptionally high, and for this reason one might conclude that the soil was unproductive. Just the reverse is true, however, for the soil was very fertile, and even with its low nitrogen and organic content produced excellent crops. Soil no. 3 was the same type of soil as no. 2, but had received fifteen tons of manure annually for the last fifteen years.

Number of organisms

The various quantities of sodium arsenite were added from a standard solution to 100 gm. portions of the different soils. These

were incubated at 28°–30° C. for ten days and then plated on synthetic agar having the following composition: distilled water, 1000 cc., dextrose, 10 gm., dipotassium phosphate, 0.5 gm., magnesium sulphate, 0.2 gm., powdered agar, 20 gm. Dilutions were made of 1 to 20,000 and 1 to 200,000, incubated for seven days, and then counted. No attempt was made to differentiate bacteria from fungi, but all were listed together as total numbers of colonies.

The ammonifying powers of the soils were determined by incubating 100 gm. of the soil with 2 gm. of dried blood to which the requisite quantity of sodium arsenite had been added. The moisture was kept at optimum and at the end of four days the ammonia determined. The nitrifying powers were determined in a similar manner, except that the samples were incubated twenty-one days and the nitrates determined by a modified Ulsch method (11). The numbers of organisms found in the three different soils with and without sodium arsenite are given in table I. The results as reported are the averages of four or more determinations. The untreated sandy soil contained 1,000,000 microorganisms which were capable of developing on synthetic agar. After one part per million of arsenic was added to this soil the number was doubled. By the time 110 parts per million of arsenic had been applied, the bacterial numbers had reached 4,000,000. At the highest concentration tested the numbers had increased eight times.

The unmanured loam receiving no arsenic contained even fewer bacteria that would develop on synthetic media than did the sandy soil. The application of sodium arsenite to this soil greatly increased the total numbers, so that by the time 200 parts per million had been applied to the soil the bacterial numbers had been increased thirty times.

The manured soil untreated with arsenic contained many more organisms than did the unmanured soil. The increase in number due to the application of sodium arsenite was about the same as the increase in the sandy soil. In the sandy soil there was an increase of 800 per cent due to the arsenic, and in the manured soil an increase of nearly 700 per cent. The unmanured soil gave an increase much greater than either of the others. The nature of the curves which one obtains from the plotting of these results is

quite similar in all three cases. There is an upward trend in all, which is very great in all cases where approximately 100 parts per million of arsenic had been applied to the soil. There is no downward trend in any of the curves, even when 200 parts per million of soluble sodium arsenite had been applied. This raises the question as to how much sodium arsenite may be applied to these soils

TABLE I

INFLUENCE OF SODIUM ARSENITE UPON NUMBER OF BACTERIA IN SOIL (10 DAYS)

TREATMENT ARSENIC (P.P.M.)	NUMBER OF BACTERIA DEVELOPING UPON SYNTHETIC AGAR		
	Sandy soil	College farm soil unmanured	College farm soil manured
None	1,000,000	900,000	3,900,000
1	2,200,000	4,600,000	3,400,000
10	2,000,000	5,000,000	4,000,000
20	2,100,000	6,800,000	5,600,000
30	2,200,000	7,800,000	5,800,000
40	2,200,000	7,800,000	7,200,000
50	1,800,000	7,600,000	7,000,000
60	1,600,000	7,800,000	7,200,000
70	1,500,000	9,000,000	7,400,000
80	1,800,000	9,400,000	7,800,000
90	1,900,000	13,200,000	9,800,000
100	2,700,000	13,400,000	11,000,000
110	4,000,000	13,200,000	12,600,000
120	4,200,000	13,000,000	13,200,000
130	4,400,000	13,000,000	13,000,000
140	8,200,000	17,200,000	13,200,000
150	5,000,000	16,200,000	14,400,000
160	5,600,000	17,400,000	14,600,000
170	6,200,000	17,800,000	18,200,000
180	6,600,000	21,400,000	19,200,000
190	7,100,000	26,000,000	25,400,000
200	8,000,000	27,600,000	26,000,000

before it would become toxic. It may be that the water-soluble arsenic does not rapidly increase in any of the soils, as there would undoubtedly be reactions between the calcium, iron, and other constituents, with the formation of difficultly soluble arsenic compounds and the production of sodium carbonate, which in a measure may account for the increased number. This, however, does not account for the great increase noted.

The results do not appear to be explained by assuming, as do RUSSELL and HUTCHINSON (18), that the soil population is complex,

and that some of its numbers act detrimentally on the bacteria, and that these are more readily killed than the bacteria. We have found no initial decrease as is the case with heat and volatile antiseptics. Moreover, one would expect that if the limiting factor be protozoa, the increase would be much more rapid in the manured than in the unmanured soil, but this is not the case. It would appear, therefore, that the arsenic is a direct protoplasmic stimulant which for a time increases bacterial growth. One of us (**8**) has shown that in pure cultures the metabolic activity of *Azotobacter* is increased for a time. This increase is followed by a negative phase which is similar to the negative phase following the use of a protoplasmic stimulant. Probably if the bacterial flora of these soils were followed long enough they would pass back to normal, and for a time might even show a negative phase.

Ammonification

The results obtained for the same soils in ammonification tests are given in table II. These also are the averages of four or more closely agreeing determinations. The toxicity of sodium arsenite regularly increases in the sandy soil, so that when the arsenic applied has reached 80 parts per million, the ammonia found after the lapse of four days has been reduced to one-half normal. When 200 parts per million of arsenic was applied there was only 34 per cent as much ammonia as in the untreated soil. The results for the college loam are similar, except that the toxicity of the arsenic does not increase as rapidly, and it is not until 200 parts per million of arsenic is applied that ammonification is reduced to one-half normal. On the other hand, the manured college loam shows first a stimulation and then a less rapid increase in toxicity. These are the only results which could be interpreted in the light of the protozoan theory. It would seem that the increase of the organic colloidal material in the soil decreases the toxicity of the soluble sodium arsenite, through the formation of a colloidal arsenite which may be less soluble than is the sodium arsenite. If this is the case, we should probably find that the toxicity would return as the organic manures decayed.

It is interesting to note that sodium arsenite has increased the total number of bacteria from eight to thirty times, and only in

four concentrations, and that only in the heavily manured college loam did the ammonifying powers increase. It cannot be argued that the ammonifying powers have been increased, and to a still greater extent the organisms which feed upon ammonia increased, as the greatest increase in numbers is not found in those soils which exhibit the greatest decrease in ammonia production. It would appear that if the arsenite has increased the number of

TABLE II

INFLUENCE OF SODIUM ARSENITE UPON AMMONIFYING POWERS OF SOIL

TREATMENT ARSENIC (P.P.M.)	MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL		
	Sandy soil	College farm soil unmanured	College farm soil manured
None	136.4	101.0	91.8
1	109.8	85.0	99.2
10	109.8	86.0	102.1
20	95.5	84.0	104.7
30	80.9	88.4	96.5
40	83.3	93.5	81.6
50	83.3	85.7	74.1
60	83.3	97.6	72.4
70	66.6	88.4	69.0
80	70.0	95.7	68.3
90	57.1	93.1	71.0
100	56.7	94.1	68.3
110	52.7	82.9	69.0
120	49.3	77.8	69.3
130	50.6	68.6	74.1
140	47.9	68.3	72.7
150	47.6	66.3	69.3
160	47.9	71.0	68.4
170	47.2	63.5	68.5
180	47.2	62.2	56.7
190	46.9	60.8	65.9
200	46.9	48.9	63.9

ammonifying organisms of these soils, it has decreased their physiological efficiency to a still greater extent. If it has not increased the total number of ammonifiers in these soils, it makes one ask what groups were increased. GREEN and KESTELL (12) found that members of the *subtilis* group were very sensitive to sodium arsenite, whereas members of the *putidum* group were very tolerant, some of them growing in one per cent arsenite broth. Arsenites, therefore, may decrease multiplication in a species with a high physiological efficiency and accelerate it in a species with a low efficiency.

Sodium arsenite is more toxic to the ammonifiers than is sodium arsenate. For example, one part per million of arsenic in the form of sodium arsenite stimulates ammonification in the college loam. This stimulation continues until seven parts per million of arsenic in the form of sodium arsenate has been applied, whereas sodium arsenite is toxic even when one part per million is applied. In this regard the bacteria act similarly to the higher plants, that is, they are more sensitive to arsenites than to arsenates, provided the cation in combination with the arsenic is not toxic. For instance, zinc arsenite (7) is less toxic than is copper arsenate, but sodium arsenite is more toxic than is copper arsenate. If we compare the less soluble compounds, arsenic trisulphide and lead arsenate, the difference is still greater. It is conceivable that the toxicity of the arsenites may decrease after they have been in the soil some time, due either to the oxidation of the arsenite to the arsenate or through the bacteria developing a tolerance for the same (17).

Nitrification

The results for the nitrification tests are given in table III. These also are the average of three or more closely agreeing determinations. In none of the soils tested was sodium arsenite a stimulant to the nitrifiers, unless it be assumed that the great increase in numbers is due to organisms which utilized nitrates, thus preventing their accumulation. If this were the case, we should expect the greatest decrease in nitrates where the greatest increase in number is found. This is not the case. It appears, therefore, that there is no stimulation of nitrifiers by sodium arsenite. On the other hand, sodium arsenate (7) when applied in the proportion of 85 parts arsenic per million of soil increased the accumulation of nitrates in this soil by 50 per cent. Furthermore, lead arsenate, Paris green, zinc arsenite, and arsenic trisulphide all act as direct stimulants to the nitrifiers. Sodium arsenite was more toxic in the sandy soil than in the college loam, as was also the case with the ammonifiers. It thus appears that the organic colloids act as a protection to the nitrifiers as well as to the ammonifiers. Sodium arsenite is much more toxic to the nitrifiers than are the arsenates. In this respect the ammonifiers and nitri-

fiers are similar to the higher plants, as KNOP (**15**) found that arsenites were much more toxic in water cultures to maize plants than were arsenates. This is also true when the plants are grown in sand (**21**).

The increase in numbers without an apparent increase in the ammonifying and nitrifying powers of the soil raises the interesting question as to what species are increased. There is the possibility

TABLE III

INFLUENCE OF SODIUM ARSENITE UPON NITRIFYING POWERS OF SOIL

TREATMENT ARSENIC (P.P.M.)	MILLIGRAMS OF NITRIC NITROGEN FORMED IN 100 GM. OF SOIL		
	Sandy soil	College farm soil unmanured	College farm soil manured
None	25.9	65.8	71.4
1	19.6	62.3	69.3
10	18.9	60.9	67.2
20	18.9	60.2	66.5
30	18.2	55.2	63.0
40	18.9	51.7	63.0
50	18.2	49.5	58.1
60	16.8	49.5	56.7
70	18.9	49.5	54.6
80	15.4	47.4	49.7
90	14.7	48.1	49.7
100	13.3	46.7	49.7
110	13.3	43.9	46.9
120	11.9	44.6	45.5
130	11.9	43.9	42.7
140	10.5	43.9	43.4
150	11.9	43.9	42.7
160	10.4	43.2	41.3
170	10.5	42.5	41.3
180	9.8	42.5	40.6
190	9.8	38.9	41.3
200	9.8	38.9	39.9

that the number of ammonifiers is increased and their physiological efficiency decreased. DANBENY (**3**) watered barley plants with a solution of arsenious acid (1 once in 10 gallons) five times in succession, and found that the crop arrived at maturity about a fortnight earlier than the untreated part of the crop, although the amount harvested was rather less. This may be what has happened with regard to the bacteria. The arsenite has hastened maturity and has correspondingly decreased the physiological efficiency, or, the

arsenite may accelerate multiplication in the less efficient groups and retard it in species having a high physiological efficiency.

KNOP considered that the stimulation of plants by arsenic was due to its replacing in a measure phosphorus. STOKLASA (**20, 21**), however, found that arsenic was unable to replace phosphoric acid, the plants dying in the flower in the absence of the latter. The senior author (**8**) has shown that arsenic cannot replace phosphorus in the nutritive media of the *Azotobacter*. It may, through a chemical interchange with the insoluble phosphorus of the soil, render phosphorus soluble, but if the *Azotobacter* is seeded into silica sand, in which the phosphorus of the Ashby media has been replaced by arsenic, there is no nitrogen fixation. Furthermore, the results point to the conclusion that the arsenic is a direct protoplasmic stimulant which shows first an increase in activity, followed by a negative phase. The stimulation of higher plants by arsenates (**19**) but not by arsenites (**1**) is what we could expect from these results. Ammonification, nitrification, and azofication are largely increased by the application of arsenates to a soil; hence the available nitrogen would be proportionally increased for the growing plant. Furthermore, the increased bacterial activity would yield increased quantities of various acids, which in turn would react with the comparatively insoluble phosphorus-carrying and potassium-carrying minerals of the soil and render them available to the plant.

Summary

1. Sodium arsenite applied to a soil greatly increases the number of organisms in that soil which will develop on synthetic agar.

2. In only three dilute concentrations were the ammonifiers stimulated by the addition of sodium arsenite to a soil. The soil in which stimulation was noted was a calcareous loam high in organic content. If the number of ammonifiers in a soil are increased by the addition of sodium arsenite to a soil, their physiological efficiency is reduced to such an extent that there is a decrease in the accumulation of ammonia over that found in an untreated soil.

3. Sodium arsenite is toxic to nitrifiers when added to a soil in only one part per million, whereas even 85 parts per million of arsenic in the form of sodium arsenate stimulates.

4. Sodium arsenite is less toxic to ammonifiers and nitrifiers in a loam soil than in a sand, and still less toxic in an organic loam than in a silt loam. The organic colloid probably forms a loose chemical combination with the arsenic, thus protecting the bacterial flora against its action.

5. Although comparatively large quantities of arsenates may accumulate in a soil without injuring its beneficial microflora, only small quantities of sodium arsenite may accumulate without producing ill effects.

AGRICULTURAL EXPERIMENT STATION
LOGAN, UTAH

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PREFERENTIAL FERTILIZATION IN OENOTHERA LAMARCKIANA

HUGO DE VRIES

In the cultures of mutant races of *Oenothera Lamarckiana*, one of the most common difficulties lies in the deviations shown by the splitting phenomena from the ordinary rules. After the self-fertilization of a splitting race, or after a cross, the expected types appear, as a rule, but in unexpected numerical relations. According to the individual cases, this may be due either to a differential growth ratio of the germs within the ripening seeds, or to preferential fertilization.

The former cause prevails among crosses of the mutants *O. gigas* and *O. semigigas*, but the second seems to be of more general occurrence. The latter may depend upon differences in individual vigor of the pollen grains as well as of the ovules. Some kinds of pollen will produce tubes with a rapid growth, whereas other tubes will have a slower development, as has been shown by the researches of NILSSON (1) and RENNER (2). Some ovules may attract the pollen tubes more effectively to their micropyle than others, and this supposition may explain the deviations in those cases in which homogeneous pollen is used.

We will first consider the cross between *O. Lamarckiana* mut. *velutina* (*O. blandina*) and *O. Lamarckiana* itself, as described by the writer (3). *O. blandina* has only one kind of gamete, belonging to the type of *O. velutina*, but modified in different points, and especially in having no lethal factor. The cross was made in 1913, the first generation yielding, as was to be expected, about one-half of the type *O. laeta*, the other half belonging to the pattern of *O. velutina*. Some of the hybrids of the first group were self-fertilized. After the rule for monohybrid splittings, we should expect the progeny to consist of 25 per cent *laeta*×*laeta*, 25 per cent *blandina*×*blandina*, and 50 per cent *laeta*×*blandina*. The first combination, or *O. amphilaeta*, must result in barren seeds, on account of the

lethal factor of the *O. laeta* gametes. Thus the ratio of the culture, resulting from the cross, would become two-thirds hybrids of the *O. laeta* type and one-third *O. blandina*. Instead of this, 33 per cent *O. laeta* and 67 per cent *O. blandina* were counted.

In order to explain these unexpected figures, we may assume that the pollen and the ovules of the race of *O. blandina* are more vigorous than those carrying the *O. laeta* gametes in *O. Lamarckiana*. After fertilization the *O. blandina* tubes will come first into the ovary, and the *O. blandina* ovules will attract them vigorously. The first copulations will produce almost purely *O. blandina* germs, and only later will the remainder embrace the other possible combinations also. It is evident that such a process may be influenced strongly by external conditions, and therefore that we must expect the deviations to be very variable in amount.

In analogous crosses of *O. blandina* this type not rarely reaches about 90 per cent of the living progeny of the hybrids, and this holds good for almost all the experiments made with this race (**4**). Whenever the differences between the expected forms among the progeny of a cross are such as to be obvious in the seed pans or in the seedling boxes, the superfluous specimens of *O. blandina* may be pulled out before transplanting into the garden, but in other cases they may considerably reduce the number of plants of the desired type on the beds, if space is limited.

The same predominance of *O. amphivelutina* fertilizations is observed in those cases where a lethal factor is present, killing the germs within the seeds. The number of barren grains must then be ascertained. *O.* mut. *oblonga* and *O.* mut. *albida* may be chosen as examples. Their formulas are (*oblonga'*+*velutina*)×*velutina* and (*albida'*+*velutina*)×*velutina*. From this we should expect about 50 per cent of barren grains in both races, after self-fertilization. The figures obtained from the harvest of 1913 and 1921 for biennial *O. oblonga*, however, were 57–62–70–82–83, giving an average of 71 per cent barren grains. For the annual plants of the same race (1911 and 1914) it was still higher. I found 67–75–94–94–95, or together 85 per cent. *O. albida* is a race of weaker constitution, and as a rule gives a still poorer harvest. I counted 84–87–89–99, or an average of 90 per cent empty seeds. So it is in numerous

other instances, the *O. amphivelutina* germs occurring almost always in a greater quantity than would be expected.

On the other hand, *O. amphilaeta* germs are usually produced in too small numbers. It may suffice to give the figures for three of the newest races, the formula of which is (*mutant'*+*laeta*)×*laeta*, which would give 50 per cent of living germs reproducing the race, and 50 per cent of *laeta*×*laeta*, resulting in barren grains. The observed figures, however, were for *O. Lamarckiana* mut. *nitens* 20–35 per cent, for *O. Lamarckiana* mut. *distans* 23–32 per cent, and for *O. Lamarckiana* mut. *elongata* 31 per cent. From this we may conclude that the *O. laeta* ovules were fertilized in these cases in too small numbers. The same condition prevails in other instances. If the pollen of these races is brought on to the stigma of other types, having two kinds of ovules, the *O. amphilaeta* will also be in the minority. For example, they were reduced to 3 per cent in *O. Lamarckiana*×*elongata*, to 15 per cent in *O. oblonga*×*elongata*, and to 11 per cent in *O. oblonga*×*nitens*.

From these considerations it is clear that, in order to be compared with the relations derived from the general rule for monohybrid splitting, the figures found for the fertilization of gametes of the type of *O. velutina* should be diminished, whereas those for the group of *O. laeta* should be increased. Constitutional strength of the races used, individual vigor, and the conditions of the cultures seem to be the chief causes which determine for each case the amount of the deviation.

Besides these, internal and hereditary factors may play some part in producing this phenomenon. Especially it may be presumed that the lethal factors might be a cause of a weaker constitution, and thereby influence the degree of the deviations from the numerical expectations. In order to try this, I have chosen a hybrid race which has only gametes of the type of *O. laeta*, but some modified and the others normal, the latter having the ordinary lethal factor, whereas the first have lost it.

In a previous article (**5**) I have described the hereditary behavior of *O. rubrinervis*. It is a half mutant from *O. Lamarckiana*, in which the *O. laeta* gametes have been modified in such a way as to lose their lethal factor and to obtain a factor for brittleness. In

the self-fertilization of this race these gametes produce brittle individuals almost without empty seeds, which have been the origin of a new race, called *O. Lamarckiana* mut. *deserens*. If this race is crossed with the parent species we get two kinds of hybrids, the composition of which may be expressed by the formulas *O.* (*deserens'*×*laeta*) and *O.* (*deserens'*×*velutina*), both of which are viable. To the former I have given a special name, *O. lucida* (5), since it has often been used in crosses or as a control for identifying the characters of other hybrids. This hybrid, *O. lucida*, evidently has the formula *deserens'*+*laeta*×*deserens'*+*laeta*, and therefore may be made use of for a comparison of the vital gametes of *O. deserens* with the lethal ones of *O. laeta*. In the present investigation, however, we are only concerned with the number of germs produced by these two forms in different crosses.

The cross *O. deserens*×*Lamarckiana* has given 18 per cent of *O. lucida* and 82 per cent of *O. subrobusta*, the latter representing the combination *O. deserens*×*velutina*. This obviously gives a confirmation of the preferential fertilization, among the uniform ovules of *O. deserens*, by the pollen tubes of the type of *O. velutina*. The specimens of *O. lucida* obtained in this experiment had 14–15 per cent empty seeds. Among their living progeny I counted in 1917 39–55 per cent of the parental type, and 45–61 per cent *O. deserens*. If we compare these figures with the hybrid formula, which would lead us to expect 25 per cent barren *O. amphilaeta* seeds, 25 per cent pure *O. deserens*, and 50 per cent of the hybrid combination, giving two-thirds *O. lucida* and one-third *O. deserens* on the beds, we see that the *O. deserens*×*deserens* have a marked advantage over the *O. deserens*×*laeta*, which may be ascribed to the greater weakness of the *O. laeta* gametes, female as well as male.

In the first place, I have studied the ovules of the hybrid *O. lucida*, fertilizing them by uniform pollen of different constitution. In *O. albida* the pollen is *O. velutina*, with its usual lethal factor. Combined with *O. lucida* it gives hybrids of the types of *O. Lamarckiana* (=*laeta*×*velutina*) and *O. rubrinervis* (=*deserens'*×*velutina*). *O. elongata*, which is a mutant from *O. simplex*, has only *O. laeta* gametes among its active pollen grains. It must produce barren grains of *O. amphilaeta* besides the maternal type (=*deserens'*×

laeta). *O. Lamarckiana* mut. *velutina*=*O. blandina* has uniform pollen without a lethal factor. It should give partly a *O. Lamarckiana*-like hybrid (*laeta*×*blandina*), as previously described (3), and partly *O. deserens'*×*blandina* or *O. subrobusta*. Lastly, *O. deserens* itself must reproduce from *O. lucida* partly the maternal and partly the paternal type. In every case the expectation from the formula would be for equal parts of both types.

The crosses were made in 1921 and sixty offspring were cultivated for each of them, comparing them during their whole lifetime (especially at the period of flowering and ripening their capsules) with analogous hybrids of known extraction. The types on the beds were those previously given, but the numerical results differed widely from the expectation. As is seen from table I, the culture on the bed of the second cross was a uniform one, whereas the others sharply showed their two types.

TABLE I

GAMOLYSIS OF FEMALE GAMETES OF *O.* HYBR. *lucida*

CROSS	PERCENTAGE	
	laeta'×mut.	deserens'×mut.
O. lucida×albida	5 Lamarckiana	95 rubrinervis
O. lucida×elongata	14 empty seeds	86 lucida
O. lucida×blandina	33 Lamarckiana	67 subrobusta
O. lucida×deserens	42 lucida	58 deserens
Average	22	78

It is evident that in the ovaries of *O. lucida* the ovules of the *O. deserens* type are far more richly fertilized than those containing the pure *O. laeta* gametes. The lethal factor of the latter is thus seen to be connected with a smaller degree of attractiveness for the pollen tubes, and this independently of the nature of the latter. Perhaps the amount of attracting substances secreted in the micropyle is different in the two cases.

We now come to the study of the pollen. In crosses with other species *O. laeta* and *O. deserens* both produce hybrids of the type *O. laeta*, the brittleness being recessive in the first generation. Empty seeds are not to be expected, and the cultures seem to be

wholly uniform during the first months of their development. When the beds are flowering, however, in July and August, two types become visible, although with only small differential characters. One of them is a normal *O. laeta*, exactly the same as those from the corresponding crosses with *O. Lamarckiana*. The other has narrower leaves, broader flower buttons, and a weaker stature. It embraces, obviously, the specimens issued from the pollen grains carrying the *O. deserens* gametes. I made the crosses in the same year as the previous ones, and cultivated sixty flowering offspring of each of them in 1921. The numerical results are given in table II. In this table the second row gives the figures for the

TABLE II

GAMOLYSIS OF POLLEN OF *O.* HYBR. *lucida*

	Cross	Percentage	
		sp.×laeta′	sp.×deserens′
A	O. biennis×lucida	48	52
	O. biennis Chicago×lucida	70	30
	O. Cockerelli×lucida	62	38
	O. syrticola×lucida	44	56
	Average	56	44
B	O. Hookeri×lucida	17	83
	O. blandina×lucida	5	95
	O. deserens×lucida	40	60
	Average	21	79

hybrids with the normal type of the corresponding *O. laeta* as obtained from *O. Lamarckiana*, whereas the third row embraces those with narrower leaves and broader buttons.

In group *A*, the species used for the cross were small flowered ones having small styles. In these no difference between the two types of pollen tubes is shown by the figures. In the large flowered species of group *B*, however, the difference is very obvious, since the relation is found to be about one-fourth to three-fourths instead of 1 to 1. Evidently the pollen tubes of the *O. laeta* grains are far excelled by those of the *O. deserens* pollen grains, since so many more ovules are fertilized by the latter.

The results of the tables may be summarized by saying that in the hybrid *O. lucida* (*deserens*×*Lamarckiana laeta'*), the gametes with a lethal factor are less effective in fertilization than those without that character. The lethal ovules, as well as the lethal pollen tubes, contribute a smaller part to the harvest than would be expected from the ordinary rule for monohybrid splittings. In other words, the *O. lacta* gametes are weak in fertilization, not only as compared with *O. velutina*, but also in comparison with those of their own type, which lack their lethal factor. From these facts it seems probable that the cause of this weakness is connected in some way with the presence of the lethal.

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LYCOPODIUM PROTHALLIA IN WESTERN MASSACHUSETTS

ALMA G. STOKEY AND ANNA M. STARR

In 1917 SPESSARD (4) published the first account of the finding of *Lycopodium* prothallia in America. At the same time CHAMBERLAIN (3), in connection with his account of the prothallia and sporelings of three New Zealand species of *Lycopodium*, gave a historical résumé of the literature on *Lycopodium* prothallia, including accounts of collections both in the open and of cultures. SPESSARD referred his material to five species: *L. complanatum*, *L. annotinum*, *L. clavatum*, and two which had not previously been reported, *L. lucidulum* and *L. obscurum*. Later he published a note (5) stating that the specimens which he had called *L. obscurum* were *L. complanatum*, but that in his later collections he had found undoubted *L. obscurum* prothallia. In 1922 he gave a more extended account of the prothallia of *L. lucidulum* and *L. obscurum* (6).

Since October 1920, eleven stations have been found in western Massachusetts, of which seven, established by the writers and W. P. STOKEY, will be described in this article, and four will be described by DEGENER in this issue. The seven stations are as follows:

1. *L. complanatum:* Moody Corners, South Hadley.
2. *L. obscurum* and *L. complanatum:* Island in Forge Pond, Granby.
3. *L. obscurum:* Mt. Toby, Sunderland.
4. *L. obscurum*, *L. complanatum*, and *L. clavatum:* Smith Ferry Woods, South Hadley.
5. *L. obscurum:* Upper Lake Woods, South Hadley.
6. *L. obscurum:* Aldrich Mills, Granby.
7. *L. obscurum:* Dark Woods, Granby.

These stations are from one to sixteen miles apart, and are in three quite distinct types of woods. The first station was found in October 1920, and the others during the summer and autumn of 1922. A description of each station will be given, with a discussion of the conditions which were found to prevail.

Station 1, Moody Corners, South Hadley.—A colony of more than 100 sporelings of *L. complanatum* was found on the northeast facing slope of a glacial dump above a small brook, in an area of about 1.5×8 m. They were 1–2.5 m. above the level of the water. The knoll is covered with a young growth of mixed hardwoods, with a few pines, and although the growth is rather open the ground is always shaded. The trees on the slope are *Tilia americana*, *Carpinus caroliniana*, and *Fraxinus americana*, with a few seedlings of *Betula populifolia*. The ground was but partly covered with a sparse growth of *Maianthemum canadense*, *Polygala paucifolia*, a species of grass, and small patches of mosses, *Polytrichum commune* and *Catherinea angustata*. The soil was firm and compact, consisting of leaf mold, clay, and sand to a depth of 8–10 cm., with a subsoil consisting of clay, sand, and gravel. The prothallia were found at a depth of 0.5–3.5 cm. The smaller sporelings were attached to prothallia, and some prothallia without sporelings were found also. The prothallia were small and less symmetrical than the typical form. The sporelings were slender and fragile in appearance, but apparently healthy. The nearest spore-bearing plants of *L. complanatum* were found 25 m. to the east, but these plants appeared to be too young to have been the parent plants.

Station 2, Island in Forge Pond, Granby.—This small island, which at its highest point is not more than 3 m. above the level of the pond, has a mixed growth of hardwoods, with a few old pines at the south end and a few young pines at the north end. Several thrifty patches of sporelings of *L. obscurum* were found at the north end, on the north and northwest facing slopes, where there is a growth of *Betula populifolia*, *Acer rubrum*, and *Viburnum dentatum*. White pines were growing near this zone, but no sporelings were found where there was any noticeable deposit of pine needles. The region is well shaded but rather open, so that few leaves remain on the ground. The herbaceous growth is rather sparse and consists of small patches of *Polytrichum commune*, a few plants of *Maianthemum canadense* and *Polygonatum biflorum*, and scattered tufts of a species of grass. The top soil consists of a layer of coarse sandy loam, 6–10 cm. deep. There is a sharp transition to the underlying stratum of sand. Numerous prothallia, both with and

without sporelings, were found 2–5 cm. below the surface. Only four prothallia of *L. complanatum* were found, all without sporelings, but these were larger and more symmetrical than those produced much more abundantly at Moody Corners. Only three fruiting spikes of *L. obscurum* were found on the island, but there were small patches of vigorous growth at distances varying from 2 to 6 m. from the sporelings. On the mainland, 200–250 m. from the island, there is a luxuriant growth of both *L. obscurum* and *L. complanatum*, which fruit abundantly, and a scattering growth of *L. clavatum* which was not found in fruit. Several plants of *L. clavatum* were found on the island near the patches of sporelings, but none was found in fruit and there were no sporelings of this species.

Station 3, Mt. Toby, Sunderland.—This station is on the west side of one of the smaller hills of the Mt. Toby group. The general aspect is distinctly different from that of the stations just described. There is almost no slope, no body of water, and the region has the appearance of being rather dry. The tree growth is of mixed hardwoods with a few young pines; there is little undergrowth. The soil is a rich sandy loam to a depth of 8–10 cm., with a sandy subsoil. There was a considerable deposit of dead leaves. Sporelings of *L. obscurum* were abundant, occurring in small groups, apparently limited to small shallow depressions. The prothallia were 2–6 cm. below the surface, averaging a somewhat greater depth than in the other stations. Owing to the presence of the layers of leaves, the stems of the sporelings, in addition to being unusually long, were unusually crooked. Some of the prothallia were exceptionally large, and attained a greater size than has been recorded for any subterranean species, measuring as much as 18×10 mm. Mature plants of both *L. obscurum* and *L. complanatum* were abundant in the woods but were at a considerable distance.

Station 4, Smith Ferry Woods, South Hadley.—Sporelings and prothallia of three species were found in this woods, which in general has the aspect of the Mt. Toby station. There is a young growth of *Quercus rubra* and *Q. alba*, an older growth of *Betula populifolia*, and the remains of old trees of *Castanea dentata*. There are a few young white pines. The sparse herbaceous growth in the vicinity

of the sporelings consisted of *Mitchella repens* and *Polytrichum commune*. The soil is a sandy loam to the depth of 6–10 cm., with sand beneath. Comparatively few leaves collect in the depressions, to which the prothallia seem to be limited. The first sporelings found were those of *L. obscurum*, three patches with three or four in a patch. These were in a broad depression near a ridge 1–2 m. high. The prothallia to which they were attached were growing 1–3 cm. below the surface. The prothallia and sporelings of *L. complanatum* were also found in depressions, shallower, however, and about 100 m. from those of *L. obscurum*. Fifteen sporelings were found, three of which were attached to prothallia. These prothallia were symmetrical in outline and relatively large, one measuring 11×2.5 mm. They were 2.5–4 cm. below the surface. In the sporelings found in this region the characteristic habit of *L. complanatum* was later in appearing than in those at Moody Corners, the flattening of the branch and the broadening of the leaf base not being evident until after the fourth or fifth branching, while in those at Moody Corners it usually began after the second or third branching. Two sporelings of *L. clavatum* with prothallia and two without were found about 10 m. from the patch of *L. complanatum*. These two prothallia were nearer the surface than any prothallia collected, and were barely covered by the loose leaf mold at the surface. Old plants of *L. complanatum* were fruiting abundantly 25–50 m. west of the patch of prothallia. Fruiting plants of *L. obscurum* were in the same general region, but were somewhat less abundant. *L. clavatum* does not fruit freely in this part of Massachusetts, and while many vigorous old plants were found, none were seen which were bearing fruiting spikes.

Station 5, Upper Lake Woods, South Hadley.—A patch of more than 50 sporelings of *L. obscurum* was found on the more or less level region 2–4 m. from the northeast side of the lake. The bank at this place is about 2.5 m. high. Many sporelings were attached to prothallia, and several prothallia were found which had not produced sporelings. The prothallia which were distributed over an area of 2×4 m. were found 1–3 cm. below the surface. The soil is a firm sandy loam to a depth of 7–10 cm., over a sand and gravel subsoil. The region is well shaded, chiefly by a large

Acer saccharum, but it is rather open and few leaves remain on the ground. The other woody growth is *Betula populifolia*, *Hamamelis virginiana*, and young *Quercus rubra*. The soil is partly covered with a species of grass and occasional small patches of *Leucobryum glaucum* and *Dicranella heteromalla*. A few spore-bearing plants of *L. obscurum* were found about 100 m. to the northwest, and another patch was found across the lake about 200 m. to the south.

Station 6, Aldrich Mills, Granby.—The place was investigated because it is one of the few places in this region where *L. clavatum* can be found fruiting freely. No sporelings of *L. clavatum* were found, but two sporelings of *L. obscurum* with gametophytes, and two colonies of sporelings which had lost their gametophytes were found. This station is distinctly different in aspect from those previously described, but is similar to two described by DEGENER. The sporelings were growing in a dense grove of young hemlocks on the north-facing slope 0.5–2.5 m. above a small floodplain on Bachelor Brook. The soil, which was completely bare of other plants, was covered with hemlock needles, and consisted of a loose sandy loam with considerable organic matter. There were no old plants of *L. obscurum* within 40 m.

Station 7, Dark Woods, Granby.—A few sporelings of *L. obscurum* were found in a well defined depression in a rather open grove of *Acer rubrum* and *Betula populifolia* on the edge of the Dark Woods. The herbaceous growth consisted of *Pyrola rotundifolia*, *Cypripedium acaule*, small patches of *Polytrichum commune*, and a species of grass. The depression was 0.4–1.0 m. below the surrounding level. About 15 m. away, on the opposite side of a slight ridge 0.6–1.0 m. high, there is a small but permanent pond. There were a few dead leaves on the ground, covering a thin layer of leaf mold; below this was sandy loam passing gradually into pure sand. There was less organic matter in the loam than was found in the other stations. There were only two sporelings with prothallia, and these were small; there were three sporelings which had lost the prothallium but which still had the foot. The prothallia were 0.7–1.5 cm. below the surface. Old plants of both *L. obscurum* and *L. complanatum* were fruiting about 3 m. away, and other patches were abundant throughout the adjacent parts of the woods.

Discussion

The stations fall roughly into three types: (1) a grove of mixed hardwoods on a slope or near a slope above a body of water; (2) depressions in a grove of mixed hardwoods where the general aspect is that of a relatively dry woods; (3) a grove of hemlocks.

Certain conditions were found to be similar in all three types of habitat. The top soil consists of sandy loam, usually with a large amount of humus, and it is fairly compact except under hemlocks. The presence of considerable humus is doubtless important, both in relation to the moisture content of the soil and to the development of the fungus with which the prothallium maintains a symbiotic relationship. The three species of prothallia are of the subterranean type, which contains an endophytic fungus. Fungal hyphae were usually found in great abundance in the soil in which prothallia were growing. In only one station was there any considerable deposit of dead leaves. It is possible that many layers of leaves would tend to prevent the spores from washing down into the soil to suitable depths. Where the spores are deposited in large quantity this might not make any difference, but where the spore deposit is light, as at a distance of 50–100 m. from spore-bearing plants, and in cases where the percentage of germination is only 5 per cent, as BRUCHMANN (2) reported for *L. clavatum* and *L. annotinum*, the presence of many layers of leaves might be an important factor in the distribution of prothallia.

In all the regions the variation in the level of the water table is moderate. This may be due to proximity of bodies of water, or to a favorable topography in relatively dry woods such as is afforded by depressions where evaporation is less than in the surrounding areas, and where drainage from the surrounding levels tends to maintain the water supply. In the hemlock groves the dense shade and the protection given from the wind, together with the high humus content of the soil, would make the maintenance of an adequate moisture content a fairly simple problem. One explanation of the comparatively rare occurrence of prothallia and sporelings may be that, owing to their very slow growth, there is always a high degree of probability that an exceptionally dry summer will lower the water table to such an extent that prothallia and spore-

lings in all stages of development will be killed. It seems not improbable that crops of sporelings would be more likely to appear after a period of 10 to 12 years without a protracted drought.

All the stations were in well shaded regions. The slopes were mostly north-facing. SPESSARD found in Michigan that prothallia and sporelings grew in the open. In western Massachusetts, however, no sporelings were found in the open, and it is not usual to find old plants in sunny places. In this region there appears to be some agreement between the conditions which are favorable for the early stages of the white pine and for *Lycopodium* prothallia. The pines which were found in almost every station agree roughly in age with the *Lycopodium* sporelings, if we may assume that the prothallia and sporelings in this region develop at a rate similar to that which BRUCHMANN determined. The presence of white pines among second growth timber was found to be a good indicator. No sporelings were found in a pure stand of pine.

In all the regions studied the soil is well drained, the subsoil consisting of sand or sand and gravel. This seems to be of considerable importance, as it was found that when sporelings and prothallia were brought into the laboratory and greenhouse, the sporelings and old plants would live for many months in undrained dishes, but the prothallia would last only a few weeks under such conditions. Prothallia, however, will live in the laboratory indefinitely if kept in well drained sandy soil. They grew particularly well on the surface of sandy loam under a bell-jar. Under such conditions they develop chlorophyll. While old plants are frequently found in swampy places, the indications are that they do not start there.

The prothallia and sporelings are restricted to places where there is little or no herbaceous growth. They are often found near small patches of *Polytrichum*, but they do not seem to be able to compete with old established patches. They usually grow at some distance from old plants of *Lycopodium*, as has been noted by BRUCHMANN (1) and SPESSARD (6), although SPESSARD reports finding a few sporelings which were growing among old plants. In only two cases were prothallia and sporelings found within 3 m. of old plants; in most cases they were 15–50 m. away, and in some cases 100–200 m. away. Nothing was found which suggested the growth of

sporelings in groups of four, as reported by SPESSARD, nor anything to indicate any other medium of spore dispersal than wind. In the case of station 5, which is within 5 m. of a much used path, it is possible that fruiting spikes may have been carried there by persons, but in the cases of stations 1 and 2, where the sporelings are even more abundant, it seems highly improbable. It is easier, naturally, to find sporelings when they are in groups, but so many isolated sporelings and groups of two or three were found that no basis is seen for any generalization about grouping.

Until we know something about the character of the endophytic fungus in relation to its occurrence in the soil, we are much restricted in our explanations of the conditions necessary for the development of *Lycopodium* prothallia. We do not know how many species there are, and whether they are of rare or frequent occurrence. If, as SPESSARD suggests, the various prothallia are associated with different species of fungi, we may have an explanation of the fact that in only one station, no. 2, were the prothallia of two species found closely associated. In stations 3, 5, and 7 the conditions apparently were equally favorable for the presence in the soil of spores of *L. complanatum*, and in station 6 much more favorable for *L. clavatum* than for *L. obscurum*. It may be that it is the absence of the fungus which makes the development of prothallia and sporelings a rare occurrence, but it seems equally probable that the rarity is due to the numerous contingencies of an exceptionally long infancy.

It is hoped that this analysis of the stations found in western Massachusetts may give some indications of the conditions necessary for the development of prothallia and sporelings, and also some suggestions as to situations in which one may look for them with reasonable prospect of success. It should not be assumed, however, that the requirements for germination and development will always be found under the same topographical conditions. In higher latitudes and in regions with fewer sunny days in summer, it is to be expected that sporelings will be found in places with less shade than those here described. While the moisture relations are undoubtedly complex, SPESSARD'S concise directions to look for prothallia in conditions less extreme than those in which the

parent plants are growing, is in line with the observations in this region.

In view of this successful search for prothallia and sporelings during the past year, it seems probable that they are not so rare as has been supposed. It is somewhat difficult to recognize the sporeling in the early stage, and they are apt to be found in what ordinarily would be considered poor collecting grounds. The chief difficulty seems to be in finding the first one. The first patch was found more or less by accident, although we had been looking for sporelings casually for the past ten years. After seeing the sporelings growing and noting the conditions which seemed to favor their development, it was comparatively easy to find other stations. It seems probable that in any place where *Lycopodium* fruits abundantly there is a fair chance of finding prothallia and sporelings if attention is given to selecting suitable places for investigation.

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FOUR NEW STATIONS OF LYCOPODIUM *PROTHALLIA*[1]

OTTO DEGENER

(WITH PLATES XI–XIII AND TWO FIGURES)

On March 29, 1922, while on a geological trip on East Rock Mountain, Great Barrington, Massachusetts, the writer noticed a few small lycopods growing in the shelter of a bowlder. Since these plants did not appear to be like any of the common lycopods in the region, they were removed and found to be sporelings, several of which still had the gametophytes attached. Unfortunately time did not permit a thorough investigation. The slope of East Rock where the specimens were found had been stripped of timber several years previously, so that bramble thickets had had time to form among the stumps and old laurel bushes. The soil from which the plants were taken was a medium sandy loam, thinly covered by a moss. There was little moisture in the soil, although the slightly overhanging rock no doubt hindered the drying influence of the sun except during the morning. The area that was turned over to get the plants might easily be covered by the hand. There were seven specimens, four of the sporophytes being still firmly connected with the gametophytes, and three showing only the disintegrating foot with which they had absorbed nourishment from the sexual plant.

The gametophyte strikingly resembles a carrot in shape. It is roughly conical, not counting the small crown at the top where the sexual organs are located. It is dirty gray in color and exhibits a pubescence due to rhizoids. The smallest specimen unearthed had a prothallium 2.5×7 mm., while the sporophyte had just barely reached the surface of the soil 15 mm. above. The root, arising from the sporeling above its foot, had divided three times

[1] In December 1922, after this paper had been written, the writer found hundreds of prothallia and sporelings of *L. cernuum* near the active crater of Kilauea, Hawaii. Since these plants grew under very unusual conditions, it is hoped to deal with them in a separate paper.

dichotomously, even though it was no longer than the prothallium itself. Another gametophyte, more nearly the size of the other two, measured 4×10 mm. Its sporeling, which in this case had just produced two equal branches, was 30 mm. in length. There appeared to be a main root from which secondary roots had developed, through a modified dichotomy. This gametophyte was buried approximately to the same depth as the one just described.

The remaining sporelings lay at a depth of about 3 cm. One of these was creeping just below the surface. Its prostrate stem showed no sign of changing its horizontal course, in spite of the fact that three upright shoots, each about 2 cm. in length, had formed. Another sporeling, the gametophyte of which had decayed, showed no marked creeping tendency, but had produced five branches within a distance of 2 cm. above the foot. Yet another, having two small shoots near the foot, had developed a main stem 5 cm. long. Higher up it was approaching the mature sporophyte in appearance, making the species determination certain.

The leaves of this sporeling, for instance, are scalelike at the subterranean base. A little farther up the terete stem they become awl-shaped, sometimes over 4 mm. in length, and arranged in whorls of threes that are spaced at intervals of about 2 mm. After the stem has branched six or seven times, the leaves, which are now four-ranked, assume two shapes. The lateral are more flattened, while those at right angles to them resemble more closely small juvenile leaves. The stem now appears laterally compressed because of the two ranks of wide leaves which possess decurrent adnate bases. This fact, in addition to the shape of the gametophyte attached to the other sporelings, proves the specimens to be *L. complanatum*. Because of a question raised by SPESSARD[2] in regard to the position in which the prothallia are found, it is well to emphasize the fact that the Great Barrington specimens grew with the longer axis inclined between the horizontal and the vertical positions.

On May 7, R. F. MARTIN, searching for *Lycopodium* prothallia at Orient Springs, near Amherst, Massachusetts, was rewarded by the discovery of three sporelings, one of which had an excellent gametophyte at the base. These plants were growing in a hemlock

[2] SPESSARD, E. A., Prothallia of *Lycopodium* in America. BOT. GAZ. **63**:71. 1917.

grove in moderately dry soil on the side of a ravine. Since many mature sporophytes of *L. clavatum* were growing near that spot, he inferred that his specimens belonged to the same species. Professor STOKEY identified them, however, as *L. obscurum*, the gametophyte of which had been discovered in 1917 by SPESSARD.

In an entirely different locality at Amherst on June 13, the writer noticed several more sporelings of a lycopod, with gametophytes. These were identified as *L. obscurum* var. *dendroideum*, identical with those found by MARTIN. This last station is situated on a steep slope covered with large hemlocks that produce enough

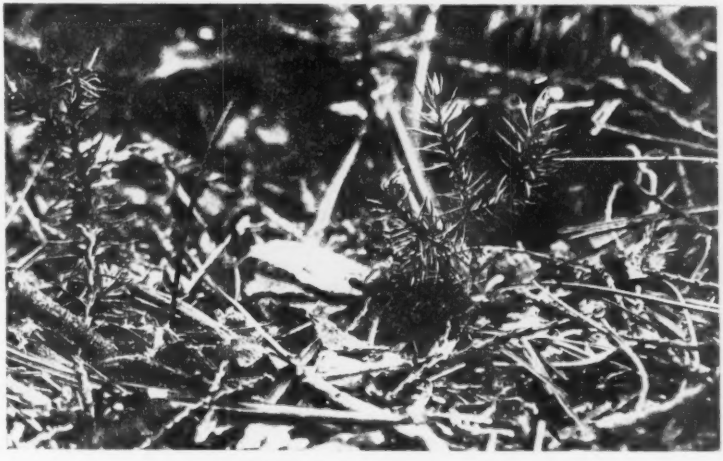


FIG. 1.—Sporelings of *L. obscurum* in hemlock grove

shade to keep the ground quite bare of plants. The soil is a moderately rich loam, underlaid at 6–8 cm. by a well drained yellow soil of similar texture. The upper layers seemed rather compact and did not crumble when dug, owing to the presence of fungal threads. Another fact possibly worthy of mention is the presence of many hemlock rootlets, the tips of which were greatly swollen with a mycorrhiza. A fungus which is probably a species of *Tremellodendron* grows well at that station. In fact, a large sporeling was found entirely surrounded by this fungus. Whether any of these have an endophytic connection with the lycopod is not known (fig. 1).

During the few afternoons that were available, between two and three hundred specimens were collected, varying from prothallia of about 1.5 mm. in diameter to mature ones with sporelings 10 cm. or more in length. Almost all of them were collected in a circular space less than three feet in diameter. More than two-thirds of the prothallia, according to measurements based on the sporelings, had developed at a depth of 2.5–7 cm. Many were growing at greater or lesser depths; only one, however, was noticed

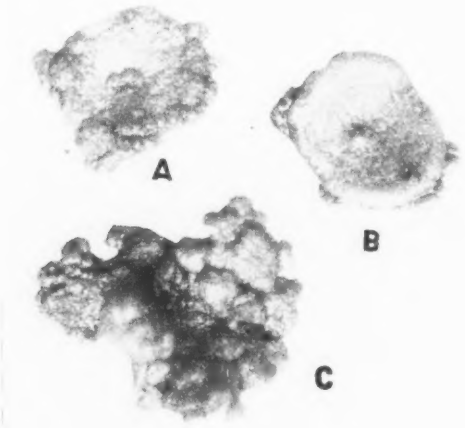


FIG. 2.—Prothallium of *L. obscurum* showing slightly depressed upper surface (*A*), prothallium showing under surface with protuberance (*B*), prothallium showing upper surface with revolute margin (*C*); ×4.5.

at 9 cm. and but a few at 1.5 cm. Because of the difficulty of distinguishing prothallia from soil particles, these figures as to the extremes of position are not as reliable as might be desired.

These plants have the saddle-shaped prothallium characteristic of such species as *L. annotinum* and *L. clavatum*. Their general contour is circular, but this shape is often modified by a later growth of unequal magnitude. The center is slightly depressed (fig. 2*A*), while in older specimens it may even be overlapped by the revolute margin upon which the sex organs are situated (fig. 2*C*). These develop intramarginally, the outer boundary of the prothallium

being well defined from the smoother sterile underside. In most of the specimens a protuberance is discernible in the center of the lower surface; it is no doubt the earliest part of the gametophyte formed (fig. 2*B*). As in the case of the specimens first described, rhizoids are visible to the unaided eye. The color, however, is of a more yellowish tinge. Fuller studies on this species, with special reference to the stelar structure of the sporeling, are now in progress.

In taking the greatest diameter of 140 prothallia from which no sporelings had developed (pl. XI), it was found that about sixty measured 4–5 mm. in the greatest diameter, half that number 6–7 mm., while seven were 8 mm. and three were 9 mm. Only two were 1.5 mm. and eight were 2 mm. in diameter. The smallest gametophyte producing a sporeling was 4 mm. in diameter and the largest 10 mm. The most frequent size, however, ranged from 5 to 8 mm.

It may be well to describe how the material was procured, for this may explain why the smaller gametophytes seemed so rare in that locality. After carefully clearing away the covering of hemlock needles and twigs from the soil, a sporeling with the soil surrounding it was removed. Then the earth was broken away from the stem of the sporophyte until the prothallium was laid bare. During this process, several other prothallia might come to view. After most of the sporelings had been gathered by this method, a handful of soil was taken from the general region and placed in a sieve to be washed. After all the earth had been removed, sometimes as many as six gametophytes were to be found among the rootlets and other particles of fibrous matter remaining. That so few small specimens were found is due no doubt to the large mesh of the sieve employed. The number of gametophytes of all sizes still growing in that locality must mount up into the thousands.

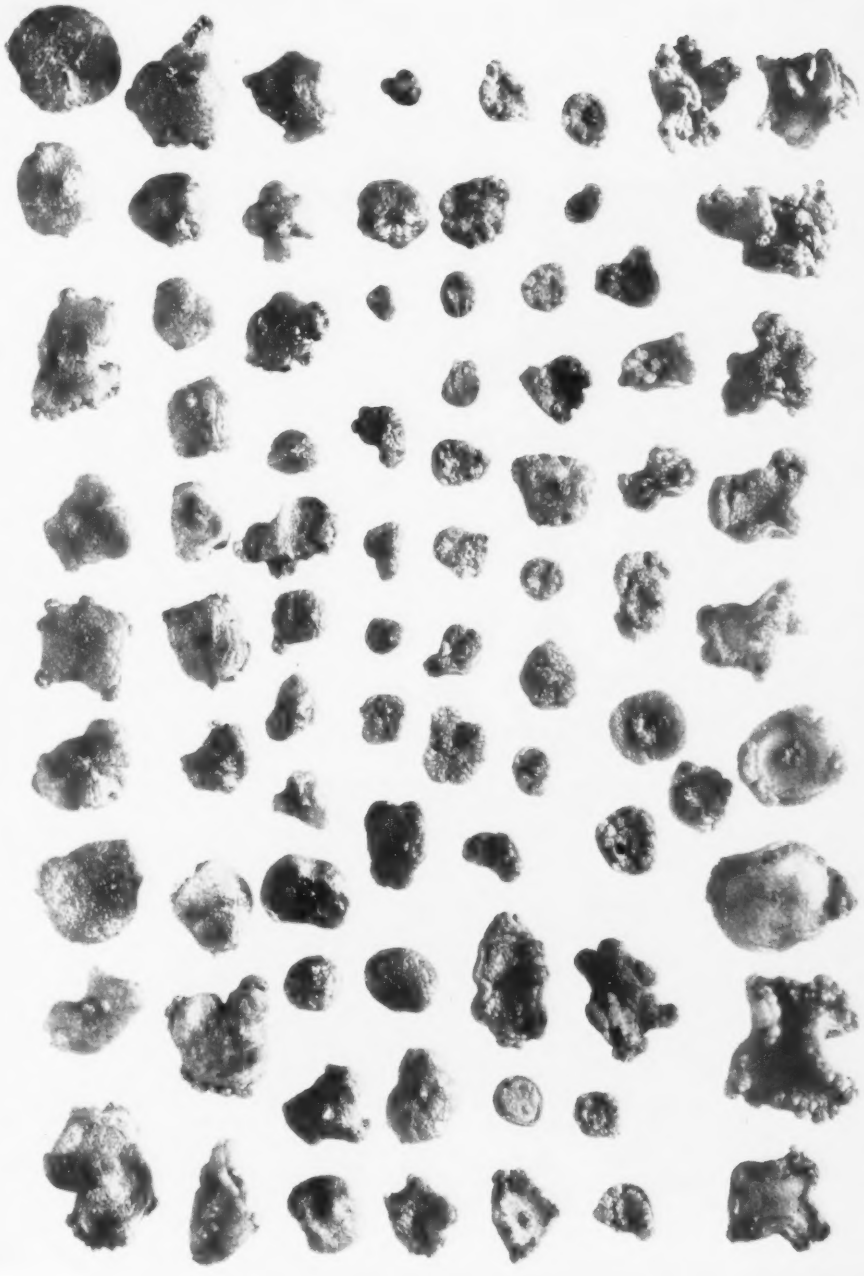
The sporelings of *L. obscurum* are upright in habit, and often produce one or two subterranean shoots which appear arrested in growth (pl. XII). The main stem commonly branches two or three times before reaching a height of 5 cm. As in the preceding species, the earliest leaves below the surface of the soil are scalelike, while the later ones are linear awl-shaped, and generally spaced farther apart on the stem than the mature type of leaf.

In both of these species the gametophyte is not restricted to growth in a definite position. As already noted, the conical gametophytes of *L. complanatum* found in Great Barrington grew neither strictly upright nor strictly horizontal in the soil. Of the specimens of the same species found by SPESSARD, one was definitely known to have grown with the longer axis in a vertical position, while others grew with the longer axis horizontally directed. In mentioning a later find,[3] he definitely states that they do not all grow in the same position. Of the few saddle-shaped gametophytes of *L. obscurum* seen by the writer *in situ* at Amherst, several were growing in an inverted position. Of the sporelings that were gathered, four had developed from inverted prothallia 6–7 mm. in diameter (pl. XIII *A*, *C*), while one had grown from a gametophyte 4 mm. in diameter which was standing on its edge so that the flattened surfaces stood vertically (pl. XIII*B*). It is probable that the surface with the reproductive organs is normally directed upward, but that when displaced by any chance, a sporeling can still develop, provided fertilization occurs.

A dozen sporelings of *L. clavatum* were later discovered growing under an isolated hemlock, possibly 400 yards from the preceding station. But one specimen, 2 cm. in length, had a saddle-shaped gametophyte 4.5 mm. in diameter attached to it. This was buried at a depth of 5 mm. The others possessed a foot about 1 mm. in diameter through which the sporeling had been attached to the prothallium. In measuring the sporelings, it was evident that the prothallium in no case grew at a depth below 2 cm.

This location is very similar to the one in which the *L. obscurum* material was discovered. The ground was not sloping, however, and was sparsely covered with moss and grass. The sporelings also were growing in close proximity to one another. In the field they were easily distinguished from the sporelings of *L. obscurum* by their prostrate habit and more fuzzy appearance, since the leaf is tipped by the bristle so characteristic of that species. The subterranean and later juvenile leaves from a macroscopical examination did not differ essentially from the corresponding types described.

[3] SPESSARD, E. A., Prothallia of *Lycopodium* in America. BOT. GAZ. **63**:362. 1918.



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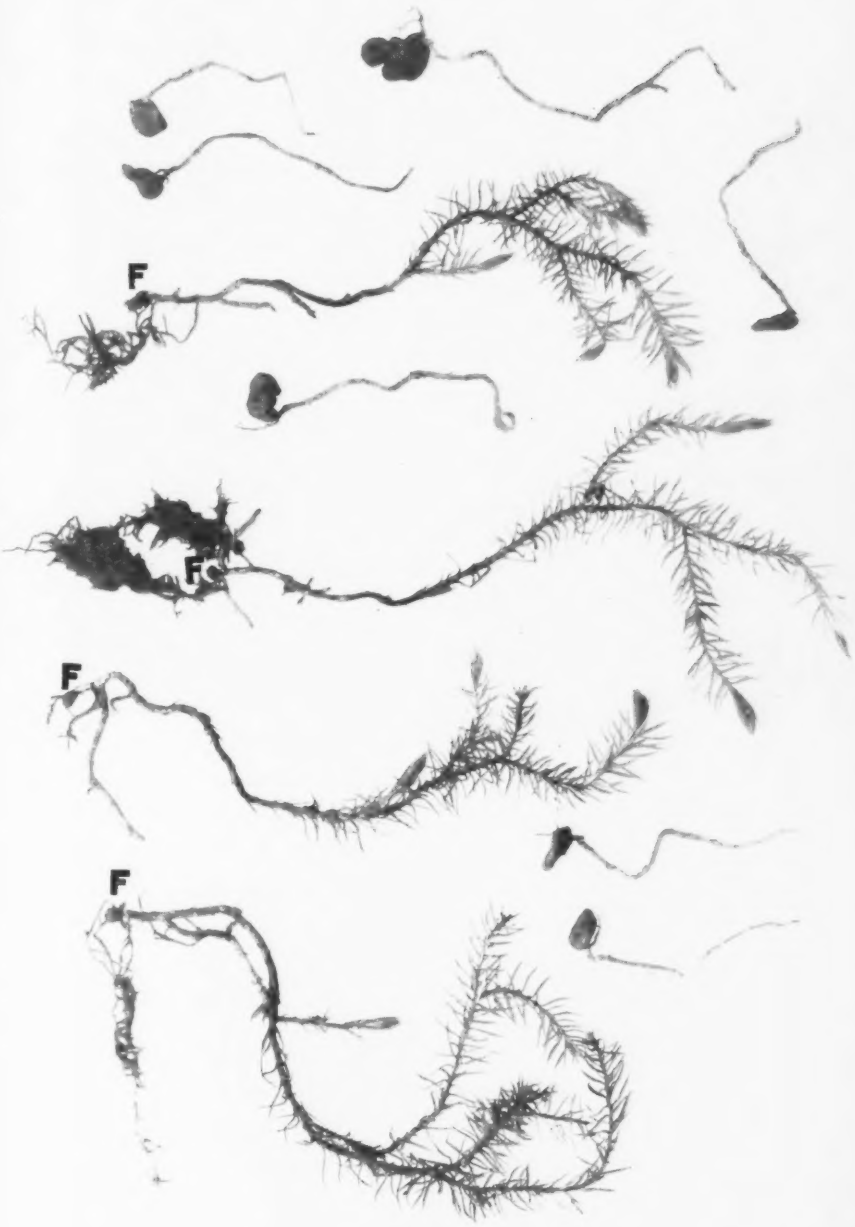

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DEGENER on LYCOPODIUM

The writer wishes to express his indebtedness to Dr. JAMES B. POLLOCK, Exchange Professor from the University of Michigan, for invaluable suggestions and criticisms in the preparation of the manuscript. Thanks are due DR. WILLIAM H. DAVIS for aid in illustrating this paper.

Summary

1. The gametophytes of *L. complanatum*, *L. obscurum* var. *dendroideum*, and *L. clavatum* have been discovered in three different stations by the writer; the gametophyte of *L. obscurum* var. *dendroideum* has been discovered at another station by R. F. MARTIN. Several hundred prothallia of *L. obscurum* were discovered in a single station.

2. The commonest depth of growth for the gametophyte of *L. obscurum* is 2.5–7 cm.; the commonest diameter of the gametophyte without sporeling is 4–5 mm., and the largest diameter is 9 mm.; the commonest diameter with sporeling is 5–8 mm., the smallest is 4 mm., and the largest is 10 mm.

3. The gametophyte of *L. complanatum* can grow and produce a sporeling whether lying with the reproductive surface in a vertical position or in a horizontal position facing upward in the soil. The gametophyte of *L. obscurum* can likewise grow and produce a sporeling whether lying with the reproductive surface in a vertical or in a horizontal position. If in the latter, it may be facing either upward or downward in the soil.

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EXPLANATION OF PLATES XI–XIII

PLATE XI

L. obscurum: four rows on left show lower surface of prothallia; the rest show upper surface; × about 2.5.

PLATE XII

L. obscurum: four largest sporelings, from which prothallium has decayed, show foot (*F*); other sporelings are still attached to prothallium; slightly enlarged.

PLATE XIII

L. obscurum: A, sporeling developed from inverted prothallium that has decayed; *B*, sporeling developed from prothallium with reproductive surface standing vertically; *C*, sporeling developed from inverted prothallium; five prothallia with revolute margins; × about 2.5.

QUANTITATIVE ESTIMATION OF CHLORIDES AND SULPHATES IN EXPRESSED PLANT TISSUE FLUIDS[1]

ROSS AIKEN GORTNER AND WALTER F. HOFFMAN

In a comprehensive study of the chemical and physico-chemical properties of plant saps as related to their environment, the question often arises as to the relative importance of the organic and inorganic constituents of the sap. This is particularly true when plants growing on alkali soils are under investigation. HARRIS, GORTNER, HOFFMAN, and VALENTINE,[2] in studies carried out in the Tooele Valley of Utah during the summer of 1920, observed that the leaf tissue fluids of perennials of that region increased in osmotic concentration with the progress of the season, indicating a response on the part of the plant to progressive desiccation of the soil. They observed, however, that all of the physico-chemical properties of the saps did not change in the same ratios, and that some plants apparently increased their osmotic concentration by a marked accumulation of the inorganic constituents in the sap, while others appeared to hold the inorganic constituents practically constant and to hold in their leaves soluble carbohydrates. The former behavior is that of typical halophytes, while the latter is more or less characteristic of those plants which grow in soils having a low salt content.

The accumulation of solutes in a sap may reach exceedingly high osmotic concentrations. HARRIS, GORTNER, HOFFMAN, and VALENTINE[3] report values equivalent to 153 atmospheres in *Atriplex confertifolia*, and 169 atmospheres in *A. Nuttallii*, both typical halophytes. Inasmuch as many alkali soils contain chlorides and

[1] Published with the approval of the Director as Paper no. 370, Journal Series, Minnesota Agricultural Experiment Station.

[2] Unpublished.

[3] HARRIS, J. A., GORTNER, R. A., HOFFMAN, W. F., AND VALENTINE, A. T., Maximum values of osmotic concentration in plant tissue fluids. Proc. Soc. Exp. Biol. Med. **18**:106–109. 1921.

sulphates, it would appear desirable to estimate both of these ions in a study of the inorganic constituents of sap from plants growing upon such soils. It may be that halophytes differ among themselves in their ability to take up these ions, just as halophytes and non-halophytes differ in their accumulation of inorganic salts in general.

Methods which have been suggested for the determination of chlorides and sulphates in plant materials do not well adapt themselves to a study of a large number of plant saps. The ideal method must meet the following conditions: (1) it must possess a high degree of accuracy; (2) it must require a minimum of material; (3) it must be rapid; and (4) it should not require complicated apparatus or exceptional skill on the part of the analyst. If possible it should be adapted to field laboratory conditions. During the past decade the biological chemists have made remarkable improvements in the methods for blood and urine analyses, all of which have been directed toward the simplification of procedure, use of small samples, and extreme accuracy. We believe, therefore, that some of these methods might advantageously be applied to plant saps, and the following methods have been found to fulfil all of the requirements.

Experimentation

DETERMINATION OF CHLORIDES.—The methods for the determination of chlorides in biological fluids are numerous. They fall roughly into two classes: (1) the destruction of the organic matter by an oxidizing agent such as nitric acid, or by charring and leaching of the chloride followed by complete ashing and then determining the chloride in the ash; and (2) the precipitation of the protein and the determination of the chloride in the filtrate.

Those falling in the first class are very time consuming, and in the case of oxidizing with nitric acid it is necessary to add an excess of silver nitrate before oxidizing to prevent a loss of chlorine. This is inconvenient, especially in the case of plant saps where the chloride content is not even approximately known, for in many cases it is necessary to "back titrate" a large excess of silver nitrate. In the case of ashing in a muffle, extreme precautions must be taken to prevent loss of chlorides.

The determinations depending upon the precipitation of the proteins are much simpler and less time consuming. There are many methods for precipitating the proteins, but the method used by WETMORE[4] for the determination of chlorides in blood appeared to be the most applicable to plant sap. In this method the proteins are precipitated by copper hydroxide, as described by HARDING and MASON,[5] and the chlorine determined by the procedure used by RAPPLEYE.[6] This method, with slight modifications, was used for determining chlorides in plant sap. The procedure adopted is as follows: 10 cc. of plant sap is placed in a 50 cc. volumetric flask, 5 cc. of 8 per cent copper sulphate, 3 cc. of a normal solution of sodium hydroxide, and enough water to make a total volume (in the flask) of about 35 cc. are added. The proteins are coagulated by heating the flask in a boiling water bath for one minute. The flask is shaken to insure complete precipitation of the protein. The flask and contents are cooled in running water, made to volume, and thoroughly mixed. The precipitate is separated by centrifuging, and the clear liquid is then decanted into a dry centrifuge tube containing 1 gm. of dry calcium hydroxide, thoroughly mixed, and again centrifuged. The treatment with calcium hydroxide serves to remove any coloring matter or excess of copper hydroxide. The chlorine is then determined by pipetting 10–20 cc. of the clear supernatant liquid into a porcelain casserole, adding 5 cc. of concentrated nitric acid, an excess of standard silver nitrate, and stirring until the silver chloride separates. Two cc. of ferric alum solution is then added, and the excess of silver nitrate titrated with standard potassium thiocyanate solution. The first reddish tinge is the end point.

To calculate the amount of chlorine, subtract one-half of the volume of potassium thiocyanate used from the number of cubic centimeters of silver nitrate added, and multiply this by the aliquot used for the titration. The number of cubic centimeters of silver

[4] WETMORE, A. S., Determination of chlorides in blood. Jour. Biol. Chem. **45**:113–118. 1920.

[5] HARDING, V. J., AND MASON, E. H., The estimation of chlorides in body fluids. Jour. Biol. Chem. **31**:55–58.

[6] RAPPLEYE, W. C., A simple application of the Volhard principle for blood plasma chlorides. Jour. Biol. Chem. **35**:509–512. 1918.

nitrate used multiplied by 0.004 gives grams of chlorine in a 10 cc. sample of sap. The reagents required are: (1) 8 per cent copper sulphate solution; (2) a normal solution of sodium hydroxide; (3) calcium hydroxide powder; (4) concentrated nitric acid; (5) a 10 per cent solution of ferric alum; (6) standard silver nitrate, made by dissolving approximately 20 gm. of silver nitrate in one liter of water and diluting until 1 cc. is equivalent to 1 cc. of potassium chloride containing 0.004 gm. chlorine per cc.; (7) potassium thiocyanate solution, made by dissolving approximately 5.5 gm. potassium thiocyanate in one liter of water and diluting until 2 cc. are equivalent to 1cc. of silver nitrate solution.

It is necessary to determine the amount of chlorine obtained on a "blank," using only the reagents. If this is significant, it must be subtracted from the total chloride content. Most of the "analytical" reagents on the market are sufficiently pure to use without making this correction. By this method good checks were obtained on duplicate determinations of chlorine in the centrifuged sap of squash leaves. Sodium chloride was quantitatively recovered after its addition to the sap, as shown in table I.

TABLE I

GRAMS OF CHLORINE RECOVERED FROM 10 CC. SQUASH SAP TO WHICH VARYING AMOUNTS OF SODIUM CHLORIDE HAD BEEN ADDED

Chlorine added	Chlorine recovered	Theoretical chlorine present	Difference	Chlorine recovered per liter	Theoretical chlorine per liter of sap
None.....	0.0064	.....	.....	0.64	.....
0.0050.....	0.0123	0.0123	0.0000	1.23	1.23
0.0118.....	0.0187	0.0182	+0.0005	1.87	1.82
0.0235.....	0.0291	0.0299	−0.0008	2.91	2.99
0.0471.....	0.0538	0.0535	+0.0003	5.38	5.35
0.0706.....	0.0761	0.0770	−0.0009	7.61	7.70
0.0942.....	0.1010	0.1006	+0.0004	10.10	10.06
0.1177.....	0.1228	0.1241	−0.0013	12.28	12.41
0.2943.....	0.3019	0.3007	+0.0012	30.19	30.07
0.3536.....	0.3591	0.3600	−0.0009	35.91	36.00

We recently had occasion to determine the amount of chlorine present in a sample of sap obtained from the leaves of *Atriplex Nuttallii*. Using this method we found 84.23 gm. of chlorine per liter. A sample of the sap was then dried and carefully ignited in platinum in a muffle, at a temperature not exceeding 500° C.

A light gray ash, free from carbon, was obtained. A chlorine determination on this ash indicated 83.52 gm. of chlorine per liter of sap, or 99.15 per cent of the chlorine indicated by the proposed method. This is regarded as proof that all of the chlorine is accounted for in the proposed method, for the actual figures obtained (2.5 cc. of sap were used in the actual titration) were 0.2105 gm. chlorine by the proposed method and 0.2088 gm. chlorine in the ash. The difference between these values is well within the experimental error.

This method is simple and takes but a short time to complete. With sufficient apparatus, a large number of determinations can be made in a day. The method is admirably adapted for carrying out the chloride determinations in a field laboratory. If it is impractical to do this, the samples may be preserved by pipetting portions of the sap into heavy walled tubes, adding a drop of formaldehyde, and sealing off the tubes with a flame.[7] Samples prepared in this manner may be preserved indefinitely. A satisfactory method of opening such sealed tubes is to heat the tube by looping around it near the top a piece of high resistance (Nichrom no. 30) wire, which is then heated red hot by an electric current controlled by a rheostat. After the tube becomes hot, the wire is removed and a drop of water placed on the heated circle on the glass, which produces a crack in the tube so that the top can easily be removed. The contents of the tube is then poured out and the tube washed until all of the contents are removed.

DETERMINATION OF SULPHATES.—The methods employed for the determination of sulphates, including unoxidized sulphur, are all based on the destruction of the organic matter and precipitation of the sulphate as barium sulphate. They depend upon the destruction of the organic matter by oxidation, using sodium peroxide, nitric acid, or some similar oxidizing agent. These methods are either disagreeable or very time consuming.

For the determination of the sulphate in plant sap, it was thought that the BENEDICT-DENIS method[8] as used by the biological chemists

[7] This method of preserving samples of sap is not original with us; Dr. J. ARTHUR HARRIS has employed it for several years.

[8] BENEDICT, S. R., The estimation of total sulphur in urine. Jour. Biol. Chem. **6**:363–371. 1909. DENIS, W., The determination of total sulphur in urine. Jour. Biol. Chem. **8**:401–403. 1910.

for determining sulphur in urine might satisfactorily be used. The method is simple, rapid, and does not require elaborate apparatus or special technique. The procedure as we have used it is as follows: 5–10 cc. of centrifuged or filtered sap is placed in an 11.5 cm. porcelain evaporating dish. Ten cc. of the BENEDICT-DENIS reagent,[9] which is ample for 10 cc. of sap, is added to the contents of the evaporating dish. This mixture is then evaporated to approximate dryness on a water bath,[10] and then carefully ignited, at first over a small flame and finally to dull redness for a few minutes, thus destroying the last traces of organic matter. After the dish cools, the ignition residue is dissolved with the aid of heat in dilute hydrochoric acid. The sulphate is then precipitated and weighed as barium sulphate. It is necessary to make a "blank" determination of the reagent and to subtract this from the total weight of barium sulphate. By using high grade chemicals a blank of 2.8–3.5 mg. of $BaSO_4$ for 10 cc. of the reagents is obtained. It is desirable not to have a blank of more than 4.0 mg. for 10 cc. of the reagent, especially when the determinations are to be made on a small amount of sap containing but little sulphate.

For the most satisfactory results the amount of barium sulphate obtained should be about 0.2 gm., but good results have been obtained when the amount was as low as 0.025 gm. and as high as 1.0 gm. These values cover a range of approximately 1 gm. to 40 gm. SO_4 per liter of sap. When the amount of sulphate is above 15–20 gm. per liter, 2–5 cc. of the sap would be sufficient for a very accurate determination. The difference between duplicate determinations is very small, usually not over 1 or 2 mg. $BaSO_4$, which is less than 0.1 gm. of sulphate per liter.

To test the accuracy of this method, a series of determinations was made on centrifuged cabbage sap. The sulphate was determined in 10 cc. of sap, and 10 cc. of sap plus varying amounts of potassium sulphate. The results of these determinations, given in table II, show that the method is accurate for plant sap. The rather large values for sulphate in the original cabbage sap probably

[9] This reagent is made by dissolving 25 gm. of crystalline copper nitrate, 25 gm. of sodium chloride, and 10 gm. of ammonium nitrate in enough water to make 100 cc. of solution.

[10] If the dishes are removed from the water bath before completely dry and the ignition carried out within a short time, very little if any spattering occurs.

are not due to inorganic sulphates, but rather to organic sulphur compounds such as mustard oils and sulphur containing proteins. It is well known that volatile oils of the Cruciferae contain organic sulphur. Such sulphur would be oxidized to sulphate by this procedure, and it would be impossible to distinguish between organic and inorganic sulphur by the proposed method. We believe, however, that the amount of organic sulphur will always be low in plant saps (with the possible exception of those plants having

TABLE II

GRAMS OF SULPHATE RECOVERED FROM 10 CC. CABBAGE SAP TO WHICH VARYING AMOUNTS OF SULPHATE HAD BEEN ADDED

SO_4 added	SO_4 recovered per 10 cc.	Theoretical per 10 cc.	Difference	SO_4 recovered per liter	Theoretical per liter
None.....	0.0336	.....	.....	3.36	.....
None.....	0.0333	.....	.....	3.33	.....
0.02.....	0.0542	0.0535	+0.0007	5.42	5.35
0.05.....	0.0835	0.0835	0.0000	8.35	8.35
0.10.....	0.1340	0.1335	+0.0005	13.40	13.35
0.15.....	0.1833	0.1835	−0.0002	18.33	18.35
0.20.....	0.2350	0.2335	+0.0015	23.50	23.35
0.30.....	0.3332	0.3335	−0.0002	33.32	33.35

sulphur compounds in their volatile oils), and need not seriously affect studies of the inorganic sulphate content of the saps. Even if organic sulphur is present in the volatile oils, the apparent sulphate content due to such sulphur will probably never exceed 5 gm. per liter of sap.

Summary

Methods have been described for the rapid and accurate determinations of chlorine and sulphates in plant saps. These methods are essentially as described by WETMORE for chlorides in blood and by BENEDICT for sulphur in urine. The manipulations are simple, and neither elaborate apparatus nor exceptional skill is required.

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RELATION OF KIND OF FOOD RESERVES TO REGENERATION IN TOMATO PLANTS

MARY ELIZABETH REID

(WITH TWO FIGURES)

A series of experiments is being carried on with tomato cuttings, to determine the relation between the character of the reserve foods which they contain and the quantity and quality of the growth response when grown under different environmental conditions, such as variations in nutrient solutions and exposure to light. Plants of two different types have been produced, according to the method suggested by KRAUS and KRAYBILL[1] in their work on vegetation and reproduction in the tomato. One type of plant, high in carbohydrates and low in nitrates, was obtained by first growing the plants to a height of 7–8 inches, and then transferring them to a nitrogen-free sand medium to which a nutrient solution lacking nitrogen was applied. The other type, low in reserve carbohydrates such as sugars and starches and high in nitrates, was obtained by growing the plants continuously in a soil rich in nitrogenous content. Exact quantitative determinations of the food reserves in the two types of plants used in these experiments have not been made up to the present time. Microchemical tests, however, indicate practically the same distribution of substances as KRAUS and KRAYBILL found in the plants with which they worked. Since they found a gradient in carbohydrate content of tomato plants increasing from the apex to the base, and a gradient in nitrate content increasing from the base to the apex, it was thought desirable to cut the longer defoliated stems into three portions, base, middle, and apex, thus by the use of these segments obtaining more closely limited relations between the nitrogenous and carbohydrate content of the stem.

Each cutting was weighed and placed in a 250 cc. test tube one inch in diameter. Two nutrient solutions (modifications of Knop's

[1] KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agric. Coll. Exp. Sta. Bull. 149. 1918.

solution) were used, one containing nitrate nitrogen and the other without nitrogen in any form. The two solutions contained approximately equal amounts of potassium, sulphur, magnesium, phosphorus, calcium, and chlorine. Nitrogen was present as an additional element in one of the solutions. These solutions were diluted with distilled water to such an extent that the total concentration of salts was 0.25 per cent in the solution containing nitrogen and 0.19 per cent in the solution without nitrogen. The solution for each cutting was replaced every four days. The original plan was to allow the cuttings to remain in the nutrient solution until the food reserves were exhausted. It was found that, in tests in which cuttings high in carbohydrates were used, from four to five weeks was the average time necessary for the fullest utilization of the food reserves present in the cuttings. In the case of the nitrogen-high cuttings it was found that the growing period was never longer than from one to two weeks for cuttings kept in darkness. If the cuttings were kept at ordinary room temperature longer than the growing period, decay set in. Since the time for the utilization of the food reserves, as evidenced by growth, was quite different for cuttings from the two types of plants, it seemed advisable to allow those from carbohydrate-high and those from nitrogen-high groups time for the fullest utilization of such reserves.

Two sets of experiments were conducted, one in the light and the other in darkness. These were repeated three different times, always with the same general results. The quantity and character of growth of the cuttings from the two types of plants were very different under the same external conditions of light, temperature, and nutrient solution. To illustrate the differences in growth obtained with cuttings of different composition, the growth responses of the basal cuttings under different conditions will be described somewhat in detail.

Experiments

BASAL CUTTINGS GROWN IN LIGHT AND IN SOLUTIONS CONTAINING NITRATES.—*Behavior of cuttings from carbohydrate-high plants* (fig. 1*A*).—These cuttings produced both roots and shoots very abundantly. Nearly all the buds which were present when the cuttings were made developed into shoots. Even below the level

of the nutrient solution, and in the region of greatest root development, there was considerable growth of the buds. The leaves were

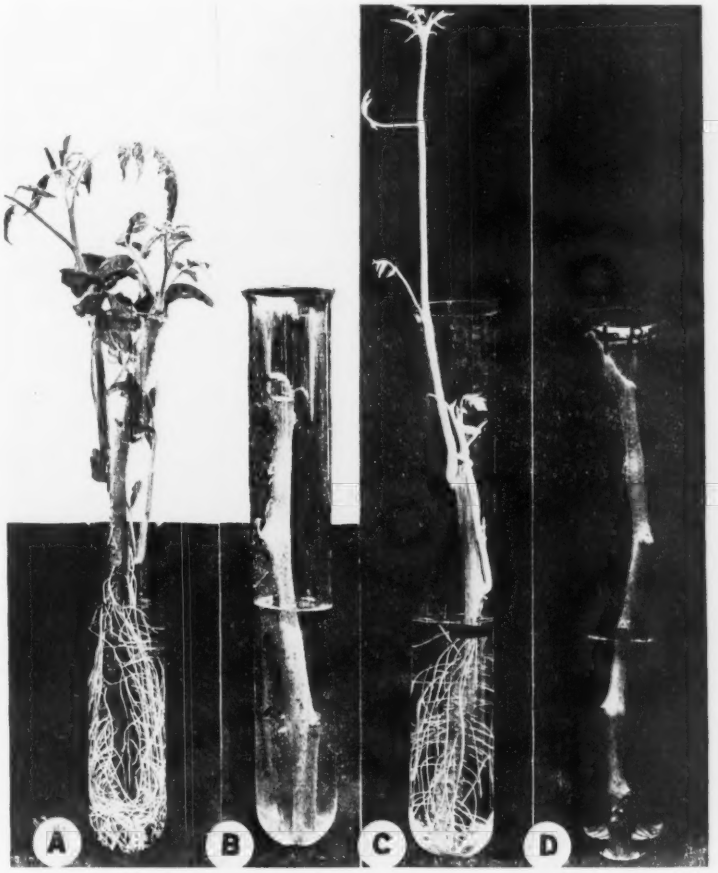

FIG. 1.—Basal cuttings grown with nitrates in nutrient solution: *A*, carbohydrate-high cuttings grown in light; *B*, nitrogen-high cuttings grown in light; *C*, carbohydrate-high cuttings grown in darkness; *D*, nitrogen-high cuttings grown in darkness.

large. The roots were very numerous, but were often without secondary rootlets. Each cutting became conspicuously greener during the time it was in the nutrient solution.

Behavior of cuttings from nitrogen-high and carbohydrate-low plants (fig. 1*B*).—In most cases these cuttings produced no roots whatever, but in case the roots did develop, they did not attain a length of more than three-fourths of an inch and had a dense growth of root hairs. In one lot of cuttings shoots developed at most of the nodes, but such shoots remained small and the leaves on them were abortive. In this particular lot of material the carbohydrate reserves were unusually low. In the other two lots, in which microchemical tests indicated a somewhat greater carbohydrate reserve, the shoots grew longer, and developed full sized leaves, which were very soft in texture and of a uniform green color.

BASAL CUTTINGS GROWN IN DARKNESS AND IN SOLUTIONS CONTAINING NITRATES.—*Behavior of cuttings from carbohydrate-high plants* (fig. 1*C*).—The general growth responses were very like those described for similar cuttings grown in the light, except that in the dark there was produced scarcely half the total quantity of both shoots and roots produced in the light. There was also a marked difference in the distribution of shoots which grew on these cuttings as compared with that of the corresponding lot grown in the light. In the light all the buds present developed into shoots, whereas in the dark in most cases the topmost bud only developed. The shoots produced in darkness were markedly etiolated. In some cases the individual shoots attained a length of fifteen inches, whereas six inches was the maximum length attained by shoots grown in the light. The roots developed to about the same length under the two sets of conditions. Preliminary microchemical tests (made only in the last experiment) indicated the presence of a very small amount of nitrate in the basal portions of some of the young shoots developed in darkness, but no nitrates were found at their apices. In most cases no nitrates could be detected in the old cuttings at the end of the experiment, but some starch was still present. The amount of starch was roughly estimated as about two-thirds the quantity originally present.

Behavior of cuttings from nitrogen-high and carbohydrate-low plants (fig. 1*D*).—There was no growth of either shoots or roots from such cuttings. The cuttings were very susceptible to bacterial

decay. Small portions of the petiole that remained attached to the cuttings showed softening and decomposition within a very few days.

BASAL CUTTINGS GROWN IN LIGHT AND IN SOLUTIONS WITHOUT NITRATES.—*Behavior of cuttings from carbohydrate-high plants.*—(fig. 2*A*.) No shoots were produced by such cuttings, but there was in most cases an abundant root growth. Microchemical tests of the old cuttings made at the end of the growth period showed a great abundance of starch still present. Although no nitrates could be detected in these cuttings, when originally placed in the nitrate-free nutrient solution, yet after root growth had proceeded for some time, nitrates could be detected. Nitrates were found to be present in greater abundance at the bases (in the region of root growth) than at the apices of the cuttings.

Behavior of cuttings from nitrogen-high and carbohydrate-low plants (fig. 2*B*).—In one experiment some of the cuttings produced a few short, very coarse, and somewhat branched roots, while in another lot of cuttings, in which the carbohydrate reserves were lower, no roots were produced. In an experiment with the cuttings containing the least amount of carbohydrates all the buds grew to some extent, but their development was slight and in no case did the leaves fully expand. On the other hand, the cuttings containing more carbohydrates produced a greater growth of shoots, with fully developed leaves of a uniform green. The roots developed after the growth of shoots was well started. This growth of roots may have been possible because carbohydrates were synthesized by the leaves.

BASAL CUTTINGS GROWN IN DARKNESS AND IN SOLUTIONS WITHOUT NITRATES.—*Behavior of cuttings from carbohydrate-high plants* (fig. 2*C*).—The type of growth of such cuttings was practically the same as that in the corresponding lot grown in the light. The cuttings produced numerous roots but no shoots. Only about half the quantity of roots was produced in darkness as in the light. Microchemical tests showed large quantities of starch still present in the cuttings when these were removed at the end of the experiment. It was roughly estimated that about three-fourths of the starch originally present was still in the cuttings. Nitrates were

found to be present, as in the case of similar cuttings grown in the light and in like nutrient solutions.

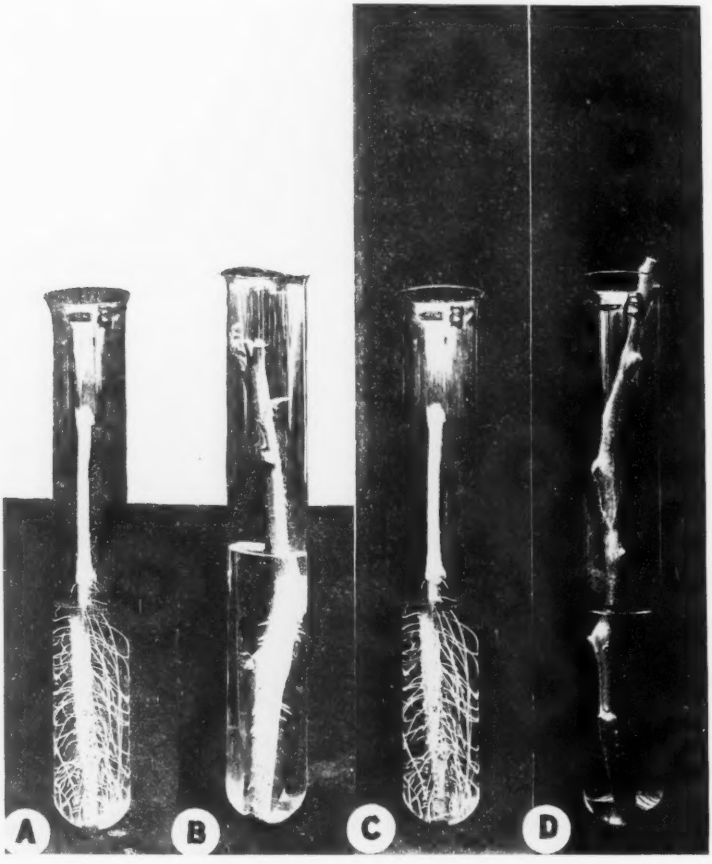

FIG. 2.—Basal cuttings grown without nitrates in nutrient solution: *A*, carbohydrate-high cuttings grown in light; *B*, nitrogen-high cuttings grown in light; *C* carbohydrate-high cuttings grown in darkness; *D*, nitrogen-high cuttings grown in darkness.

Behavior of cuttings from nitrogen-high and carbohydrate-low plants (fig. 2*D*).—In one lot of material, in which there was a small amount of reserve carbohydrates in the cuttings, there was a very

small amount of shoot growth but no root growth. In another lot of cuttings, in which the carbohydrate reserve was still lower, there was no production of either shoots or roots.

BASAL, MIDDLE, AND TOP CUTTINGS.—In the case of carbohydrate-high cuttings grown in the light without nitrates, in the nutrient solution the middle and top portions produced some shoot growth, whereas no growth of shoots occurred from the basal portions. In most experiments in which such cuttings were grown with nitrates in the nutrient solution, the total growth of roots and shoots was greater from the basal than from the middle portions, and greater from the middle than from the top portions. It should be stated in this connection that the basal cuttings had a greater average weight than the middle cuttings, and the middle cuttings a greater average weight than the top cuttings.

The vegetative cuttings, both in the solution containing nitrates and in the one without it, showed slight variations in the relative amounts of shoot and root growth by the different segments. In the cases where the relative differences between carbohydrate and nitrogen reserves in the different levels of the stem may be very slight, further experimentation will be carried on to determine more definitely the relationships of root and shoot growth.

In the case of carbohydrate-high cuttings grown in the solution containing nitrates, it was generally observed that when equal weights of cuttings from the three regions of the stems were compared, the greatest amount of shoot growth was made by the apical cuttings, whereas the basal portions produced the least shoot growth. In the case of root growth it was the basal portions which produced the greatest amount, both in number and weight of roots. The middle portions produced roots second in number and weight, and the top portions the least amount.

The roots which appeared on the middle and top cuttings in most cases were confined to a limited region around the lowest node. The roots from the middle portions were longer and more branched than those produced by the top or basal portions. In most cases the roots from cuttings grown in the nutrient solution without nitrogen remained in a healthy condition for a longer time than those grown in the solution containing nitrates. The latter frequently showed a brownish discoloration.

In the carbohydrate-high cuttings grown in the nutrient solution without nitrogen, the leaves were small, pale green, and relatively stiff in texture. In this case the nitrogen supply was limited, both within and without the plant. When there was a great abundance of nitrogen both in the plant and in the solution, but with a low carbohydrate reserve in the cuttings, the leaves were soft in texture.

Summary

1. It was very noticeable that in cuttings high in nitrogen, grown in the light in a nutrient solution lacking nitrates, considerable shoot growth resulted, and that in some cases more growth resulted than if nitrates were present in the solution.

2. The presence of nitrates in the nutrient solution in the case of nitrogen-high cuttings appeared to be unfavorable for root growth. If the carbohydrate reserves are exceedingly limited, the nutrient solution containing nitrates may also be unfavorable for shoot growth. In striking contrast with this condition, the same sort of solution favored development of shoots from cuttings high in carbohydrates. In this case root growth was also favored, although to a less extent.

3. In general, it seems that when the carbohydrate reserve is high and the nitrogen supply within the plant or in the nutrient solution is low, there may be a vigorous root growth. When the relative percentage of nitrogen either within or without is slightly higher, there may also be a vigorous shoot growth.

These investigations were carried on in the department of plant physiology at the University of Wisconsin, and helpful directions and criticisms were given by those in charge.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH
YONKERS, N.Y.

AN UNUSUAL GROWTH OF MOLD[1]

FREDA M. BACHMANN

(WITH THREE FIGURES)

A few years ago (1917) a very unusual growth of mold was found, which had formed in a bottle of grapejuice. The grapejuice had been boiled. The bottle was filled to the top of the neck with the hot liquid and immediately covered with thin cloth and sealed with sealing wax. It was stored in a cellar in which the temperature varied from about 60°F. in summer to nearly freezing in winter. The age of the culture is uncertain, but the grapejuice was bottled before the year 1900, so that when it was found, it was at least seventeen years old. The 8 ounce bottle was 9 inches high, 3 inches wide, and 1.25 inches thick. The cylindrical neck measured 3 inches in length and somewhat less than 1 inch in diameter. When I first saw it, the liquid and the mold filled the bottle to within an inch of the top (fig. 1).

Through handling, the growth became loosened from one side of the neck. Although the seal seemed perfect, a small amount of air must have entered, enough to support a slow vegetative growth of the mold. The sealing wax was very brittle, and it may be that with different changes in temperature it had a greater viscosity at times and at others more brittleness, resulting in some slight cracks.

Since the growth of the mold took place only at the surface of the liquid, the resulting mass of hyphae was shaped by the neck of the bottle into a firm cylindrical mass and forced downward. As this gradually lengthened, it must have become longer than the bottle, and, because of the narrowness of the bottle, the mold growth formed a number of folds like a letter *S*. The absence of any growth of the hyphae from the surface of the mass submerged in the liquid is a striking example of the dependence for growth of molds upon the presence of atmospheric oxygen.

[1] Published by permission of Director of Wisconsin Agricultural Experiment Station.

There was no evident change in the appearance of the growth from 1917 until about a year ago, when I decided to open the bottle and test the hyphae for viability. The seal was broken with aseptic precautions and replaced by a sterile cotton plug. Microscopic examination of the mycelium showed it to be septate. Some of the cells were without content, apparently dead cells, in others the scanty protoplasm filled only part of the cell, while in still others the microscopic picture was that of slightly plasmolyzed or even normal cells. The ends of some of the hyphae were much enlarged. Fig. 2 shows parts of a number of hyphae as they were when the bottle was first opened.

FIG. 1

A transfer of some of the surface hyphae was made to sterile potato agar. A gray-green growth resulted in a few days. The mold sporulated readily on this medium. Fig. 3 represents the conidiophores. The mold appears to be a species of *Aspergillus*. Since the seal was broken the mold has grown considerably, but it is no longer in the neck of the bottle. The liquid has evaporated, so that it is about two-thirds of an inch below the neck. The mold growth is spread out on the surface and is a much softer, looser growth. Spores have been formed in abundance.

Since it is not known how long a time was required to produce such a growth of mold, it seemed of interest to attempt to reproduce it. In March 1922, three bottles of a liquid consisting of grapejuice

diluted with a 3 per cent dextrose solution were heated in the steamer for 30 minutes. As soon as the liquid was cool enough it was inoculated with mold spores. One bottle was inoculated with a species of

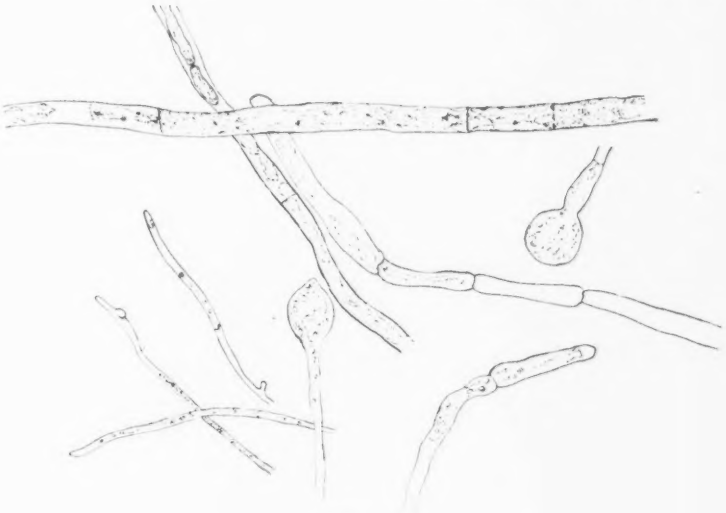

FIG. 2

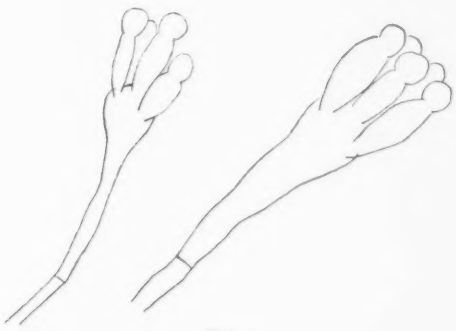

FIG. 3

Penicillium, and the other two with the *Aspergillus* isolated from the growth already described. The necks of the bottles used were not long, so that the mold growth was not a compact, perfectly

cylindrical mass, but it was shaped somewhat by the neck of the bottle. The *Aspergillus* produced growths 1 inch long, and *Penicillium* a growth nearly 3 inches long. The growth of the mold in the bottle shown in fig. 1 measures at least 14 inches in length. It did not seem to change after it was found in 1917, so that probably nearly all of this growth was before that time. The rate of growth must be influenced by the amount of oxygen present, and since it is not possible to compare the porosity of the seal of the original with that of the bottles prepared last March, no conclusions can be drawn as to the age of the culture.

If the seal had been very imperfect, there would have been considerable loss in the liquid, due to evaporation. From the compactness of the mass before the seal was broken, and the evidence showing the seal to have permitted very little passage of air, it appears that the growth must be the result of many years at least.

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BRIEFER ARTICLES

EDWARD W. D. HOLWAY

(WITH PORTRAIT)

EDWARD WILLET DORLAND HOLWAY died in Phoenix, Arizona, March 31, 1923, at the age of seventy years. He was born in Adrian, Michigan, in 1853, and when about one year old his parents moved to what were then the frontier settlements of northeastern Iowa. His boyhood years were spent on a farm near the village of Hesper. As a youth he was preparing himself for the profession of civil engineering, but was interrupted by severe illness. During his convalescence he accepted a position with the Winnesheik County Bank of Decorah, Iowa, an institution with which he remained connected for thirty-five years.

During his long and successful career as a banker he became interested in botany, eventually specializing in the Uredineae, and while still engaged in the banking business he built up a very extensive collection and valuable library along his chosen field. Wishing to devote all his time to botanical research and traveling, in 1904 he retired from active connection with the bank, and moved to Minneapolis, in order to be in closer touch with academic life. Here he presented his collections and library to the University of Minnesota, and accepted the position of Assistant Professor of Botany, which he held until his death.

Mr. HOLWAY published many papers in his chosen field, both individually and in collaboration with J. C. ARTHUR and others. His greatest

work is a monographic publication on the North American Uredineae. The first part of this appeared in 1905, and at the time of his death he had published four parts and left the completed manuscript for the fifth part, all devoted to the genus *Puccinia*.

Mr. HOLWAY was a great traveler, and not the least valuable of his scientific contributions are his collections of fungi and other plants from various little known parts of the world. While still in the banking business he traveled extensively in the United States and Mexico, and after his retirement he made several trips to Central America and western Canada, besides two long journeys in South America, the first covering the western part of that continent from southern Chile to Ecuador, the second largely confined to eastern and southern Brazil. He had just returned from the last journey when he was attacked by the illness which terminated in his death.

A man of indomitable energy, he was always fascinated by exploring little known and inaccessible regions. When nearly fifty years of age he became an enthusiastic alpinist and thoroughly mastered the art of mountain climbing. He undertook a number of difficult exploring and climbing expeditions, particularly in the Canadian Rockies, the Caribou Range, and the Selkirks. In the latter range alone he climbed twenty-three summits over 10,000 feet high, thirteen of them first ascents and nine of these without guides.

At the time of his death Mr. HOLWAY was a member of the Royal Geographical Society, the American Alpine Club, the Canadian Alpine Club, and the Society of Sigma Xi. At one time he was a member of the Botanical Society of America. He was a remarkable example of a successful business man with an essentially scientific mind, who finally turned completely away from commercial affairs to devote all his time to what he considered the more worth while pursuit of science.—F. K. BUTTERS, *University of Minnesota, Minneapolis, Minn.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Soil acidity and plant distribution.—Abstracts of a number of papers by WHERRY,[1] dealing with the problems of acidity of soils and the relation of acidity to plant distribution, are brought together and summarized in this report. Indicators to be used, methods of making determinations and for tabulation of data are suggested. Studies on ferns, orchids, and ericads are reported, with the range of acidity or alkalinity for each species, and the most frequently found, or optimum, value. It is claimed that the peculiarities of the flora of the New Jersey pine barrens, as well as certain similarities between peat bog and salt marsh border floras, can be explained on the basis of differences in soil acidity.

In a lengthy paper OLSEN[2] reports the results of studies with meadow and woodland plants on Danish soils. Acidity of the soils, as determined by an improved colorometric method, runs from P_H 3.4 to P_H 8. Within the P_H range for any given species there is a narrower range in which the species has its maximum frequency. Pot and solution culture experiments confirmed field observations, in that acid soil plants became chlorotic and were unable to make good growth in neutral or slightly acid media, while alkaline soil plants were at a decided disadvantage in acid media. Attempts were made also to study the effects of aluminum and of nitrate nitrogen as compared with ammonia nitrogen on soil acidity and plant distribution and growth. Both factors are considered to be without effect, but the experiments on which the conclusions are based are far from convincing to anyone reasonably familiar with the literature in these fields.

Both authors believe that the distribution of plant species under natural conditions varies regularly with the soil reaction. That plants differ in acid or alkali tolerance is unquestioned. That there is also an intermediate range of soil reaction, within which each species is able to make good growth when other conditions are likewise favorable, is probable. When, however, one considers the great variability in soil reaction, vertically and horizontally, met in the field, and the enormous number of physical, chemical, and biological factors involved in the growth of any individual plant, one wonders whether the

[1] WHERRY, E. T., Soil acidity: its nature, measurement, and relation to plant distribution. Smithsonian Rept. 247–268. 1923.

[2] OLSEN, CARSTEN, Studies on the hydrogen ion concentration of the soil and its significance to the vegetation, especially to the natural distribution of plants. Compt. Rend. Trav. Lab. Carlsberg **15**:1–166. 1923.

"hydrogen ion" is not being called upon to explain too much. Frequently the so-called acid plants are found growing vigorously in non-acid habitats.

It is true that each plant is a measure of the conditions, internal and external, under which it grows; but its growth is the resultant of many factors, few of which are overwhelmingly dominant. If any factor stands out clearly in world distribution of vegetation, it is water supply, not hydrogen ion concentration of soil waters. If one is trying to set sharp limitations to classes of plants as related to soil acidity, perhaps a more accurate method would be desirable. SALTER and MORGAN[3] have shown that the soil-water ratio affects the P_H value, and other factors may also affect the values obtained, as the amount of indicator used, the amount of extract tested at a time, the length of time the chemicals interact, the degree of turbidity of the soil extract, and the quality of the color chart used. Unless these are carefully standardized, potentiometric checks upon readings would be very desirable. Finally, how are we to classify plants which are sometimes found growing in soils that vary vertically by considerable P_H values? Is a plant growing on a neutral soil, with good root development extending down into an acid subsoil, to be classified as belonging to an acid habitat? When one finds the same species growing a hundred yards away on an acid surface soil with neutral or alkaline subsoil, and good root development in both, how does one arrive at a classification, or can the investigator take his choice?—R. B. DUSTMAN.

Enzymes and fungal infection.—Several years ago the discovery was made that mold spores contain enzymes. This has stimulated further research on the enzyme equipment of spores, and the possible influence of this equipment on the infection of host plants. HARTER and WEIMER[4] have demonstrated the occurrence of amylase and pectinase[5] in the spores of *Rhizopus tritici* and *R. nigricans*, regardless of the temperature at which the spores had been produced. The pectinase was used with sweet potato tissue and found to attack the middle lamella. The enzyme is more active, or more abundant in the spores of *R. tritici* than in *R. nigricans*.

Since various species of *Rhizopus* differ as to temperature requirements, the effects of temperature on pectinase production by the mycelium have been investigated[6] for nine parasitic and two non-parasitic species. The two non-parasitic species, *R. microsporus* and *R. chinensis*, both produce more pectinase than *R. nigricans*, a parasitic form. The production of pectinase is

[3] SALTER, R. M., and MORGAN, M. F., Factors affecting soil reaction. I. The soil-water ratio. Jour. Phys. Chem. **27**:117–140. 1923.

[4] HARTER, L. L., and WEIMER, J. L., Amylase in the spores of *Rhizopus tritici* and *R. nigricans*. Amer. Jour. Bot. **10**:89–92. 1923.

[5] WEIMER, J. L., and HARTER, L. L., Pectinase in the spores of *Rhizopus*. Amer. Jour. Bot. **10**:167–169. 1923.

[6] WEIMER, J. L., and HARTER, L. L., Influence of temperature on the pectinase production of different species of *Rhizopus*. Amer. Jour. Bot. **10**:127–132. 1923.

least at high temperature, 40° C., and increases with decrease of temperature to 9° C. The cell walls of old potatoes are more readily attacked than those of freshly dug new potatoes. These fungi, however, cannot penetrate the unbroken skin of the sweet potato. Infection[7] occurs through wounds, apparently only when some dead tissue gives the fungus a saprophytic start. With this start, pectinase is developed, which dissolves the middle lamella and causes cells to die, thus extending the necrotic region. Since wounding is a preliminary necessity to *Rhizopus* rot in the sweet potato, losses can largely be obviated by care to avoid injury in digging, storing, and marketing.—C. A. SHULL.

Monograph of Peronospora.—The fourth part of the fifth volume of the contributions to the cryptogamic flora of Switzerland is devoted to an elaborate study of the genus *Peronospora* by GÄUMANN.[8] The investigation extended over a period of five years and was completed in 1918, since which time it has been awaiting publication. To better institute comparisons, the species for the most part are brought together under the host families. Beside the usual technical description, synonymy, distribution, etc., almost every species is illustrated with very exact drawings of conidiospores, conidiophores, and biologic curves based on the length and the breadth of the spores. The dimensions of the spores were secured by taking the average of 500 measurements made with a Leitz graded micrometer. A large number of new species are described. Although primarily undertaken as a study of the Swiss flora, it was extended to include all known species in order to secure a broader and more accurate viewpoint. The general study is preceded by a systematic key to the Swiss species, numbering 142. The character of the oospore wall throws the species into two sub-families, and each of these into two groups. In the separation of species much stress is laid upon the size of the conidiospores. The minutiae and comprehensiveness of the study may be inferred from an introductory review of the species concept, especially as applied to the genus *Peronospora*, covering twenty-six pages, in which are inserted much experimental data. The work forms a notable contribution to cryptogamic literature.—J. C. ARTHUR.

Resin formation.—The formation of resin in conifer needles has been investigated by HANNIG,[9] who apparently overthrows TSCHIRSCH's theory of resin secretion which claimed the presence of a special resin-secreting membrane on the outside of the resin-forming cell. HANNIG is unable to find such mem-

[7] HARTER, L. L., and WEIMER, J. L., The relation of the enzyme pectinase to infection of sweet potatoes by *Rhizopus*. Amer. Jour. Bot. **10**:245–258. 1923.

[8] GÄUMANN, ERNST, Beiträge zu einer Monographie der Gattung *Peronospora* Corda. Beiträge zur Kryptogamenflora der Schweiz **5**[1]:1–360. 1923.

[9] HANNIG, E., Untersuchungen über die Harzbildungen in Koniferen Nadeln. Zeitschr. Bot. **14**:385–421. 2192.

branes, and explains how improper methods of preparation may have produced TSCHIRSCH's "Schleimbeleg," or "resinogene Schicht." The protoplasm of the epithelial cells surrounding the resin ducts is believed by HANNIG to be the source of resin formation. The resin droplets are first seen on the surface of the protoplasm, just inside the walls of the cells, only on the sides bordering on the canal. Since these droplets lie between the cell wall and protoplasm, HANNIG thinks that turgor pressure may be important in forcing the resin through the cell wall into the canal. By microchemical tests it is shown that the droplets formed by the cells and the resin in the canals are chemically alike. An excellent review of the microchemistry of resins is given. Larger droplets are found in the secreting cells of young needles than of old ones, according to HANNIG. The new HAAS and HILL text (Vol. I) states that the greatest flow of resin occurs in the spring, when, of course, the needles are young.—J. B. RHINE.

Bibliography of colloids.—An extensive bibliography of literature dealing with the colloidal state has been prepared for the National Research Council by HOLMES,[10] and published in mimeograph form. While the list is preliminary to a more complete bibliography, about 1900 titles are listed, dealing with every phase of the colloidal state. Among the classified groups are many of biological interest, such as adsorption, biocolloids, clays and soils, dialysis, emulsions, gels, gums, indicators, protein swelling, surface tension, ultra microscope, viscosity, etc. Investigators in this field will welcome the opportunity to secure accurately prepared and reasonably complete lists of citations on these topics. There is hardly a single field of science which does not at some point deal with colloidal behavior, and the National Research Council is rendering a notable service to science in having this bibliography prepared. The conservation of the time of the individual investigator is very important, and these aids to investigation materially increase the productivity in research.—C. A. SHULL.

Electrical stimulation.—A résumé of some experiments on electrical stimulation of plants is given by KOKETSU,[11] in which protoplasmic streaming is brought to a standstill, or the size of cells diminished. The responses were studied from various angles, such as rate of response, intensity effects, value of threshold stimulus, duration of required stimulus, etc. Changes in sensitivity, polar effects, galvano-tropisms, taxies, and tetanus effects were demonstrated. The author seems not to have been aware of the more extensive work of BOSE along these lines.—J. B. RHINE.

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[10] HOLMES, H. N., A bibliography of colloid chemistry. Nat. Res. Council, Washington, D.C. $1.00.

[11] KOKETSU, R., Über die Wirkung der elektrischen Reizung an den Pflanzlichen Gebilden. Bot. Mag. **36**:129–132. 1922.